Develop New or Improved Approaches for Treating Disease and Disability

IN THIS SECTION:

SCIENCE ADVANCES

Intellectual Impairment Does Not Affect Alcoholism Recovery

Background: In traditional alcoholism treatment, people learn strategies to avoid relapse. They include 12-step programs, such as Alcoholics Anonymous (AA), that focus partly on teaching new coping techniques and similar strategies. However, approximately 45 to 70 percent of alcoholics exhibit difficulties in problem-solving and memory formation. Theoretically, this kind of intellectual impairment can hinder the progress of alcohol treatment by inhibiting the learning of new ways of thought and behavior. In fact, some studies suggest that learning-impaired alcoholics do not fare well in treatment programs that are intellectually demanding.

Although most alcoholics entering treatment demonstrate normal intellectual ability, overall, results of specialized tests indicate widespread impairment of specific mental functions. These impairments include difficulties with problem-solving, abstract thinking, and formulating plans to deal with changing life situations. In addition, some alcoholics may have structural abnormalities in portions of the brain involved in goal-directed behavior and in the ability to inhibit responses involved in excessive drinking.

All of these brain functions represent targets of addiction treatment. However, no clear evidence has been offered to prove that impairment of such functions actually predicts failure of alcoholism treatment. Investigators recently examined the relationship of these intellectual impairments on the ultimate outcome of an intensive course of 12-step addiction treatment.

Advance: More than half of the participants demonstrated some form of relevant intellectual impairment, but those impairments turned out not to be a strong predictor of treatment outcome. However, cognitively impaired and unimpaired patients appeared to use different strategies for attaining abstinence from alcohol. The researchers suggested that intellectually impaired participants may have neglected more difficult approaches and concentrated on simpler strategies, such as avoiding alcohol-associated environments and seeking social support, approaches on which AA relies heavily.

Implications: These findings suggest that alcoholics with subtle intellectual deficits should not be excluded from treatment programs on the assumption that such deficits are predictors of treatment failure. In addition, researchers seeking to measure alcoholism treatment effectiveness should probably concentrate on the ultimate outcome rather than the success of intermediate steps, since different patients may arrive at abstinence through different pathways. (This does not, however, obviate the importance of understanding intermediate steps to abstinence, since researchers can design better therapies based on this knowledge.)

Morgenstern J, Bates ME: Effects of executive function impairment on change processes and substance use outcomes in 12-step treatment. Journal of Studies on Alcohol, 60(6):846-55. 1999.

Emerging New Treatments for Nicotine Addiction

Background: Nicotine addiction is at the root of one of this nation's most serious public health problems, tobacco use. Research on nicotine addiction has led to a number of effective treatments including the nicotine patch, gum, and nasal spray, all of which help smokers alleviate the withdrawal symptoms that occur when they stop smoking. Researchers continue to explore new avenues to help smokers quit.

Advance: Researchers have developed two new potential nicotine addiction treatments. Using immunological techniques, researchers have developed a nicotine vaccine that prevents the drug from reaching the brain. When injected in laboratory animals, the vaccine stimulates the immune system to produce antibodies that bind tightly to nicotine. The antibody-bound nicotine is too large to enter the brain, thereby preventing nicotine from producing its many effects. When nicotine was given to vaccinated rats, scientists found that the amount of nicotine reaching the brain was reduced by 64% and nicotine's ability to increase blood pressure and stimulate locomotor movement in rats was blocked.

Another series of studies have found that blocking the metabolism of nicotine can reduce smoking. Researchers have found that individuals with a genetic deficiency in nicotine metabolism mediated by an enzyme in the liver (CYP2A6) are less likely to start smoking, and smoke less if they do start, than individuals with normal CYP2A6 activity. Building on this knowledge, scientists tested more than 200 medications to find compounds that decreased CYP2A6 activity. They found that methoxsalen, used to treat skin disorders, significantly reduced nicotine metabolism. To look at how methoxsalen affected cigarette smoking, the researchers conducted two studies. In one study, 17 regular smokers with normal CYP2A6 metabolism received methoxsalen or placebo in combination with oral nicotine replacement. The participants who took high doses of methsoxsalen reported far less desire to smoke and had nicotine levels in their blood roughly twice as high as those given placebo or low doses of methoxsalen. By decreasing nicotine metabolism, which keeps more nicotine in the blood, smokers needed fewer cigarettes to replace nicotine lost to metabolism. In a second study, participants received either methoxsalen or placebo in combination with nicotine or placebo and after a 60-minute period of abstinence were allowed to smoke at will for 90 minutes. Smokers who received methoxsalen plus nicotine smoked fewer cigarettes, had longer intervals between cigarettes, and took fewer puffs on each cigarette.

Implications: These studies pave the way for two new nicotine addiction treatments that may help patients who aren't responding to current nicotine replacement therapies, thus offering a greater range of treatment options to individuals who want to quit smoking.

Pentel PR, Malin DH, Ennifar S, et al: A nicotine conjugate vaccine reduces nicotine distribution to brain and attenuates its behavioral and cardiovascular effects in rats. Pharmacology, Biochemistry and Behavior, 65(1):191-8. 2000.

Sellers EM, Kaplan HL, Tyndale RF: Inhibition of cytochrome P450 2A6 increases nicotine's oral bioavailability and decreases smoking. Clinical Pharmacology and Therapeutics, 68(1):35-43. 2000.

A Combination of Buprenorphine and Antabuse Appears Effective in Treating Individuals Addicted to Heroin and Cocaine

Background: More than 50% of individuals addicted to opiates, such as heroin, are also addicted to cocaine. The development of medications for treating these dual addictions is of the utmost importance. No medications have yet proven to be effective for the treatment of cocaine addiction, though there are quite a number of effective treatments for other addictions, including buprenorphine for heroin addiction and disulfiram (marketed as Antabuse) for alcoholism.

Advance: Previous research has shown that either buprenorphine or methadone alone is effective in reducing opiate use, but neither is effective in reducing concurrent cocaine use by opiate-dependent individuals. A recent study found that the participants who received a combination of disulfiram and buprenorphine abstained from cocaine use for longer periods of time than those who received only buprenorphine. These same participants also achieved three weeks of continuous cocaine abstinence sooner than those who received buprenorphine alone. No significant differences were found in the total weeks of opiate abstinence between the disulfiram/buprenorphine and the buprenorphine-only group. This research also reinforces previous studies that suggest administering disulfiram prior to cocaine inhalation may block the pleasurable and rewarding effects caused by an excessive release of dopamine in the brain after cocaine use. Instead of experiencing euphoria and other feelings of well being associated with cocaine use, an individual who has taken disulfiram before using cocaine will experience adverse reactions such as anxiety, dysphoria or paranoia.

Implications: The results are promising for the potential effectiveness of disulfiram treatment for cocaine addiction in heroin addicts who are being treated with buprenorphine. Having safe and effective medications to treat dual addictions will be beneficial to both the patient and society as a whole.

George TP, Chawarski MC, Pakes J, Carrol KM, Kosten TR, Schottenfeld RS: Disulfiram versus placebo for cocaine dependence in buprenorphine-maintained subjects: a preliminary trial. Biological Psychiatry, 47(12):1080-86. 2000.

New Behavioral Treatments for Marijuana Addiction

Background: Marijuana is the most frequently used illegal drug in the United States. Nearly 69 million Americans over the age of 12 have tried marijuana at least once. Despite the growing need for treatment for this particular drug problem, very few treatments that have been shown to be effective for marijuana dependence. Researchers are now working to see if behavioral therapies that have been shown to be effective for other drugs of abuse work for marijuana.

Advance: The researchers compared three behavioral treatments that have been shown to be effective for other drugs of abuse to see how they would work for individuals seeking treatment for marijuana addiction. The researchers found that individuals who received voucher incentives had higher abstinence rates after the 14 weeks of treatment than those who did not receive the voucher incentives. In the voucher-based incentive program, participants attended individual therapy sessions and could earn vouchers exchangeable for retail goods or services such as movie passes, sporting equipment, or vocational classes if their urine samples were determined to be drug free. The other treatments were also behavioral in nature, but did not include voucher incentives for drug abstinence. When the voucher-based incentive program was added to a behavioral intervention, marijuana abstinence improved when compared to either a motivational or a cognitive-behavioral therapy alone.

Implications: Given the results of this study, there is more science-based evidence that drug addiction treatments can and do work. This finding adds to the growing evidence that voucher-based incentive programs are an effective method for enhancing treatment outcomes. In addition, they provide treatment practitioners with a viable option for treating marijuana addiction.

Higgins ST, Badger GJ,Budney AJ: Initial abstinence and success in achieving longer term cocaine abstinence. Experimental Clinical Psychopharmacology, 8(3):377-86. 2000.

Stabilized Methadone Patients Have New Treatment Options

Background: Addiction to heroin remains a major public health problem in the United States. The good news, however, is that there are a number of effective treatments for heroin addiction. Methadone maintenance therapy is one of the most effective and well-studied treatments available for heroin addiction. It has been shown to reduce illicit opiate use, reduce crime, enhance social productivity, and reduce the spread of infectious diseases such as hepatitis and HIV/AIDS. Unfortunately, methadone maintenance therapy is only available to a small proportion of the people in the United States known to be addicted to opiates. Many federal, state and local restrictions exist for prescribing methadone. There is a tremendous need to expand both the access to and the variety of treatments currently available to opiate addicts.

Advance: A twelve-year study following patients being treated for heroin addiction, found that when physicians give a full month of take-home doses of methadone to stabilized, well-functioning patients who were already being treated with methadone, the patients do well. This monthly dosing practice, or Methadone Medical Maintenance, is an alternative for treatment of stable methadone maintained individuals. Existing regulations require all patients being treated with methadone for heroin addiction to attend the clinic one or more times a week for medication dispensing. In this study, 21 patients who had been abstinent and functioning well on methadone maintenance for extensive periods of time (average of 9.7 years) were dispensed a month's worth of take-home doses of methadone in a physician's office. The participants were evaluated once a month by a primary care physician affiliated with the methadone clinic they had been using and urine samples were collected. The results showed that only 6 patients (28.6%) dropped out during the 12 years of the study, and only 12 of 2,290 urine samples (0.5%) collected were positive for drugs. No methadone overdoses or diversion were observed. Participants reported significant improvement in their quality of life.

Implications: This study provides us with a possible scenario to expand treatment options for some heroin addicts. The expansion of medical maintenance can critically enhance patient-treatment matching by providing abstinent, high-functioning patients the least restrictive treatment necessary to maintain their improvements. The implementation of this program for stable patients will also enhance the capacity of methadone programs to admit new patients and concentrate more of their limited clinical resources on less stable patients.

Schwartz RP, Brooner RK, Montoya ID, Currens M, Hayes M: A 12-year follow-up of a methadone medical maintenance program. The American Journal on Addictions, 8(4):293-9. 1999.

Clinical Toolbox Expands for Treating Heroin Addiction: Heroin Use Reduced by Medications

Background: There are an estimated 980,000 chronic users of heroin in the United States, making opiate addiction a significant public health problem. Fortunately, three decades of NIH-supported research and clinical practice have brought the Nation a variety of effective approaches to treat drug addiction. Several effective medications have been developed to treat heroin addiction, including methadone, levomethadyl acetate (LAAM), and most recently buprenorphine (still pending final FDA approval).

Advance: When three medications for heroin addiction were compared (methadone at higher doses than normally given for maintenance, LAAM, and buprenorphine) all three markedly reduced illicit heroin use. LAAM produced the longest duration of continuous abstinence; buprenorphine when administered three times a week was similar to LAAM and high-dose methadone in retaining patients. Once buprenorphine is approved, clinicians will have three effective opioid agonist medications from which to choose when treating heroin addicts.

Implications: More treatments (medications and behavioral) are needed to ensure that practitioners have a variety of treatment options to meet the specific needs of their patients. Having the flexibility to allow eligible patients an opportunity to visit a treatment facility on a less regular basis than is currently required is likely to increase treatment retention. At individually optimized doses, LAAM, high doses of methadone, and buprenorphine, yielded similar positive outcomes, and the convenience and efficiency of thrice-weekly dosing rather than daily dosing may make LAAM and buprenorphine attractive options for treatment of heroin addiction.

Johnson RE, Chutuape MA, Strain EC, Walsh SL, Stitzer ML, Bigelow GE: A comparison of levomethadyl acetate, buprenorphine, and methadone as treatments for opioid dependence. The New England Journal of Medicine, 343(18):1290-7. 2000.

New Hormones Show Promise as a Therapeutic and Male Contraceptive

Background: The male hormone testosterone is required for producing sperm as well as for maintaining libido and secondary sexual characteristics. Lower than normal testosterone levels in men have been associated with low energy, depression, loss of lean body and bone mass, and impotence, among other symptoms. Commercial formulations of testosterone, administered through injections or skin patches, are used primarily in hormone replacement therapy for men who are producing insufficient testosterone in their testicles ("hypogonadism"). However, since testosterone is broken down rapidly in the body, these formulations last for relatively short periods of time in the bloodstream, and consequently require frequent administrations.

Advance: NIH-supported investigators recently received a patent for new androgens (hormones that produce masculine characteristics) that are considerably more potent than naturally occurring testosterone. These synthetic compounds have been evaluated in animal experiments and, if found to be safe, will be evaluated clinically. Eventually, they could be formulated into very long-acting injectable drugs and certain derivatives of the compounds may even be effective when taken orally.

Implications: Male hormone replacement therapy with testosterone is effective but is somewhat cumbersome, requiring either frequent injections or the use of a relatively large skin patch. These new compounds show promise as successful therapeutic agents that can relieve the symptoms associated with hypogonadism and are easier to administer. In addition, a serious need exists for potent androgens that can be developed into a contraceptive for men. The new androgens patented by NIH researchers, or their derivatives, may also play a significant role in developing an effective male contraceptive.

Cook CD, Kepler JA, Lee YW, Wani MC: Androgenic steroid compound and a method of making and using the same. U.S. Patent 5,952,319. 1999.

Long-Term Behavioral Effects of Iron Deficiency Anemia in Infancy

Background: Iron deficiency in infancy is a common problem which causes altered behavior and development. Approximately 20 to 25 percent of all infants in the world have iron deficiency anemia, and many more have iron deficiency without anemia. In the United States, poor and minority children are at increased risk. Overall, five percent of poor African American and Hispanic infants and toddlers, and specifically 18 percent of poor Mexican American infants have iron deficiency anemia.

Advance: NIH-supported investigators evaluated cognitive, socioemotional, motor skills and school functioning of a group of Costa Rican children between 11 and 14 years of age who had tested positive and had been treated for iron deficiency anemia as infants. Researchers compared the outcomes with a matched group of children who had good iron status in infancy. Both groups of adolescent children were free of iron deficiency and growing normally by U.S. standards; however, the investigators found that those children who had severe, chronic iron deficiency in infancy scored lower on measures of mental and motor function when tested in adolescence. Significant differences were found in arithmetic achievement, written expression, motor functioning, spatial memory, and selective recall. Twice as many of the adolescents who had been anemic infants had repeated a school grade compared to members of the nonanemic cohort and three times as many required special services. Parents and teachers rated the behavior of the formerly anemic adolescents as more problematic, especially related to anxiety/depression, social and attention problems.

Implications: Severe, chronic iron deficiency anemia in infancy has permanent effects on brain function. This nutritional anemia should be diagnosed and treated as early as possible to ensure normal brain development and function throughout life. Early screening for iron deficiency anemia is particularly important for minority infants, because of their increased risk for the condition, especially infants who are African American, Hispanic, and more specifically Mexican American.

Lozoff B, Jimenez E, Hagen J, Mollen E,Wolf AW: Poorer behavioral and developmental outcome more than 10 years after treatment for iron deficiency in infancy. Pediatrics, 105(4):E51. 2000.

Promising Target for New Drugs Against Malaria

Background: Malaria is on the rise worldwide, killing at least one million individuals a year, mostly in Africa. More than half a billion people are infected with malaria worldwide and a quarter of the world's population is estimated to be at risk for this infection. Twenty-five percent or more of infected children die every year from malaria, one child succumbing every 12 seconds. The death rate among those infected is rising because the parasite, *Plasmodium falciparum*, which causes malaria is becoming resistant to conventional therapies. As of yet, there is no vaccine to prevent malaria infection and, until one is developed, there is an urgent need for new ways to treat the disease.

Advance: When the malaria parasite enters the body it attaches itself to the outer membrane of a red blood cell and slowly works its way into the cell. About 10 hours later, after the parasite has entered the cell, it needs a food source to sustain it and help it reproduce. NIH researchers have discovered that the malaria parasite, through the use of electrically charged particles, can create a pore-like hole through the red blood cell membrane. This channel acts like a straw through which the parasite supplies itself with the nutrients and elements that it needs for survival.

To demonstrate this phenomenon, the NIH researchers applied a microscopically thin glass electrode, with a diameter of about one $100{,}000^{th}$ of an inch, to the surfaces of infected and non-infected red blood cells and compared the electrical current flowing through the cell wall in response to an applied voltage (typically one tenth of a volt). Using special amplifiers, researchers were able to detect current changes that measured as little as a few picoamperes (one billionth of an ampere). With these instruments, the researchers also determined that red blood cells harboring the parasite had over a thousand such channels, which were much larger and had an electrical charge not associated with the normal pathways found in red blood cells.

Implications: Since these voltage-dependent channels are unique to infected red blood cells, they are probably a good target for new anti-malarial drugs. Investigators are now trying to determine if the parasite creates these channels by modifying a protein in the cell membrane of its host red blood cell, or by producing a new protein within the red blood cell that is then incorporated into the cell membrane. With this information scientists could tailor drugs to cut off the parasite's supply lines without affecting the ability of a normal blood cell to transport nutrients across its membrane.

Desai SA, Bezrukov SM, Zimmerberg J: A voltage-dependent channel involved in nutrient uptake by red blood cells infected with the malaria parasite. Nature, 406(6799):1001-5. 2000.

Severe Burn Patients During Bandage Changes

Background: Approximately 50,000 Americans each year are hospitalized with serious burn injuries, according to the American Burn Association. These injuries cause extreme pain and actually change the body's chemistry, making this pain difficult to treat with drugs and other therapies. The pain is particularly acute when a patient's bandages are changed. This daily procedure often generates anxiety in patients – anxiety that itself exacerbates pain. Studies have shown that high levels of pain during hospitalization are correlated with poorer mental and physical functioning of burn patients after release.

Advance: Researchers attempted to distract teenage burn patients during daily bandage changes by immersing them in a virtual reality environment that included an interactive glove and a helmet that projected 3-D images with a wide field of view, preventing the patients from seeing the procedure. Previous studies have shown that people have only a finite amount of conscious attention available, and that feeling pain actually requires some of this attention. When patients become engrossed in activities that require a lot of conscious energy, they perceive less pain. So during daily wound care, burn patients are usually engaged in conversation, mental imagery or effort, or shown videos. In the new study, the researchers placed patients in a virtual reality environment to test whether the experience was significantly absorbing to reduce further the patients' pain during bandage changes. In fact, the patients reported a dramatic drop in their pain, anxiety, and focus on the procedure when immersed in a virtual reality game called SpiderWorld than when playing a Nintendo 64 video game. When captivated by the sights, sounds, and tactile stimulation in SpiderWorld, one patient reported spending 2% of his time thinking about his pain during a bandage change. When not playing the video game the patient spent 95% of the time focused on the pain.

Implications: Burn patients, particularly children, are often not given enough pain medication. Unfortunately, high doses of the morphine-based drugs used can cause side effects like respiratory failure, nausea, and brain disorders (encephalopathy). By reducing pain without additional medication, virtual reality could make wound care less harrowing and improve overall healing for burn patients. In addition, the use of virtual reality to absorb children and adolescent patients may be able to lower the anxiety that accompanies daily bandage changes. If the technique proves similarly successful in a larger number and greater variety of patients, it may become the newest aid to healing for burn patients – and others suffering from extreme acute pain.

Hoffman HG, Doctor JN, Patterson DR, Carrougher GJ, Furness TA: Virtual reality as an adjunctive pain control during burn wound care in adolescent patients. Pain, 85(1-2):305-9. 2000.

Living Skin Grafts Enhance Burn Treatment

Background: In the United States, 1.25 million people seek medical attention for burns every year, according to the American Burn Association. Third-degree burns, which extend to the deepest of the skin's layers, require immediate care to prevent infection and dangerous fluid loss that can lead to shock. A quarter-century ago, NIH-funded burn surgeons determined that badly burned skin should be removed as quickly as possible (rather than letting it slough off over time), followed by immediate and permanent replacement of the lost skin. This seemingly simple idea ultimately became standard practice for treating major burn injuries and led to the development of what is now an artificial skin system called Integra_. After removing the damaged skin, surgeons blanket a burn wound with a covering like Integra_, then apply a skin graft on top of this biomaterial to coax the growth of new skin to close the wound. While ideally surgeons obtain skin grafts from the burned patient, in the case of severe burns covering 80 to 90 percent of the body surface, there is not enough remaining skin to use for this purpose.

Advance: Now, burn researchers have succeeded in growing skin cells from a burned patient and adding them to a polymer sheet to create living skin grafts in the laboratory. In an effort to permanently close burn wounds, the scientists placed the laboratory-grown skin grafts on top of Integra_ and bathed everything with a nutritious mix of growth factors and antibiotics to help prod the growth of new blood vessels and control infection. The researchers tested this technique on three children who had been badly burned in fires. The results were promising, showing that the new method offers an advantage over other currently available technologies, such as using non-living epidermal substitutes that cannot as accurately restore the structure and function of native skin. In each test case, the patient's new skin was a lighter color than before, but it had returned to its original softness, smoothness, and strength – with minimal scarring.

Implications: The new method may improve the treatment of severely burned patients who have lost more than half of their skin to third-degree burns, because the availability of skin for grafting the burn wounds of these patients is often limiting to recovery. This approach to wound treatment may also decrease treatment costs and hospitalization times associated with the treatment of severe burns, though more studies are needed to formally test these predictions. Finally, the method succeeded in regaining much of the cosmetic appearance of the burn-damaged skin of these young patients – a crucial element in helping burn victims return to a normal life.

Boyce ST, Kagan RJ, Meyer NA, Yakuboff KP, Warden GD: Cultured skin substitutes combined with integra artificial skin to replace native skin autograft and allograft for the closure of excised full-thickness burns. Journal of Burn Care Rehabilitation, 20(6):453-61. 1999.

Chemists Improve Synthesis of Anticancer Agent

Background: According to the National Cancer Institute, soft tissue sarcomas – tumors of the muscles, tendons, and supportive tissues – afflict approximately 7,300 Americans each year, typically requiring surgery and radiation treatment. Chemotherapy is not a frontline treatment for most soft-tissue sarcomas because up to 40 percent of those who are treated with chemotherapy agents experience a relapse of the cancer. Additionally, the returning cancer is often impervious to the chemotherapy drugs.

Advance: Chemists have improved the synthesis of ecteinascidin, a potent antitumor roughly 100 times more powerful than Taxol_, a leading anticancer drug. Although ecteinascidin was discovered in 1988, it has not been widely available because it had to be purified from its natural source, where it exists in tiny quantities. The new synthesis makes commercial-scale production possible. Ecteinascidin is in Phase II clinical trials and has shown the ability to shrink drug-resistant soft tissue sarcomas. In addition, it may inhibit drug resistance in other forms of cancer. Other researchers have shown that ecteinascidin prevents the formation of P-glycoprotein, a protein associated with multidrug-resistant tumors. P-glycoprotein transports toxins such as chemotherapy drugs out of cancer cells, thereby preventing the drugs from destroying the tumor. Ecteinascidin stops cells from forming more P-glycoprotein, eliminating one of the cancer cells' best defenses against chemotherapy agents.

Implications: If its clinical trials are successful and it is eventually approved by the U.S. Food and Drug Administration, ecteinascidin would be the only drug available to treat those sarcoma patients in whom a prior round of chemotherapy had failed. Additionally, by preventing the formation of P-glycoprotein, ecteinascidin may keep other types of tumor cells vulnerable to chemotherapy. Even if ecteinascidin is not proven to be effective on its own, it may become a key ingredient in chemotherapy "cocktails" to prevent tumors from developing resistance to existing anticancer drugs.

Martinez EJ Corey EJ: A new, more efficient and effective process for the synthesis of a key pentacyclic intermediate for production of ecteinascidin and phthalascidin antitumor agents. Organic Letters. 2(7):993-6. 2000.

Jin S, Gorfajn B, Faircloth G, Scotto KW: Ecteinascidin 743, a transcription-targeted chemotherapeutic that inhibits MDR1 activation. Proceedings of National Academy of. Sciences, 97(12):6775-9. 2000.

New Strategy to Prevent Lung and Liver Injury in Alpha 1-Antitrypsin Deficiency

Background: Alpha 1-antitrypsin deficiency is an inherited disorder affecting 1 in 1600 live births. It is characterized by low blood levels of the alpha 1-antitrypsin protein that predispose individuals to premature development of emphysema and chronic liver disease. Although a form of the alpha 1-antitrypsin protein is produced in patients with the disorder, the mutant form folds incorrectly, causing it to be retained in the liver rather than secreted into the blood and body fluids. Because the mutant form retains about 80 percent of the function of the normal version, increasing production of the mutant version could help compensate for deficiency of the fully active, normal protein.

Advance: Researchers discovered that a compound known as 4-phenylbutyric acid (PBA) is able to increase secretion of the alpha 1-antitrypsin protein in a cell culture model. In addition, PBA increased production of alpha 1-antitrypsin in mice that had been altered to have the mutant human gene. PBA was proven to be well tolerated and without any side effects in these mice.

Implications: The only treatment option proposed for alpha 1-antitrypsin deficiency has been to replace the protein in the blood through infusions. However, this is costly and inconvenient, and the availability of protein does not meet the demand. The idea of enhancing secretion of a mutant, but partially active, protein is new and may lead to a strategy for prevention of the injuries that typically result from the disorder.

Burrows JAJ, Willis LK, Perlmutter DH: Chemical chaperones mediate increased secretion of mutant α1-antitrypsin (α1-at) z: a potential pharmacological strategy for prevention of liver injury and emphysema in a1-at deficiency. Proceedings of the National Academy of Sciences, 97(4):1796-1801. 2000.

New Ventilation Strategy Saves Lives in Patients with Acute Respiratory Distress Syndrome

Background: Acute respiratory distress syndrome (ARDS) is a life-threatening lung condition marked by inflammation and an accumulation of fluid. It occurs in conjunction with catastrophic medical conditions such as serious infections and trauma. Each year, approximately 150,000 Americans are affected and nearly 40 percent of them die. Treatment consists of supportive care, which includes use of mechanical ventilation and supplemental oxygen to help patients breathe. However, studies of this standard therapy had suggested that traditional approaches to mechanical ventilation might actually exacerbate the problem and cause further lung damage.

Advance: For the entire 30 years since ARDS was first defined, no therapy had been shown to be effective in reducing mortality. Now, a breakthrough has occurred. A new strategy for ventilator use reduced deaths by 22 percent compared with standard ventilation practices and also reduced the number of days spent on the ventilator. Investigators designed a randomized controlled clinical trial to compare the effects of standard mechanical ventilation therapy with those of a mechanical ventilation strategy that forces smaller volumes of oxygen-enriched air into the lungs of ARDS patients. The trial showed that using smaller volumes delivered by the ventilator greatly improved survival. In fact, the new ventilator strategy was so successful in reducing deaths that the trial was halted early and all patients were then treated using the newer method. Smaller volumes of air appear to improve clinical outcomes in patients with ARDS by minimizing damage to the lungs due to stretching.

Implications: Using the smaller volumes of air as a ventilation strategy will save an estimated 15,000 American lives each year and will decrease health care costs by reducing the number of days required on a ventilator in an intensive care unit.

The Acute Respiratory Distress Syndrome Network: Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. The New England Journal of Medicine, 342(18):1301-8. 2000.

Initial Results of Clinical Trial Raise Hopes for Gene Therapy

Background: Hemophilia B is an inherited bleeding disorder resulting from a deficiency of factor IX, a protein that is essential for the blood clotting process. The current state-of-the-art treatment entails regular intravenous infusion of clotting factor to prevent the crippling joint damage and life-threatening bleeding complications of the disease. Although treatments for hemophilia have become safer, therapeutic products are still not risk-free; patients with hemophilia are at risk of contracting blood-borne diseases through their numerous transfusions.

The ultimate goal is to offer a cure for the disease, and the strongest hope for a cure comes from gene therapy. For gene therapy to be feasible, however, scientists must package the factor IX gene into a carrier, or vector, that can safely transfer the gene into a patient. They also must determine how to cause the gene to manufacture adequate amounts of the needed protein once the carrier is inside a patient's cells.

Advance: After promising preclinical studies in hemophilic mice and dogs, a gene therapy clinical trial was initiated in adults with hemophilia B. The gene was enclosed in a carrier called adeno-associated viral vector, which was injected into the muscle of the patient. Results from the first three patients, who received the lowest dose of treatment, suggest that the vector may be safe and provides biologically active factor IX in the blood. Ongoing studies of these patients and of others will help to establish further safety parameters and therapeutic dosing for this gene therapy strategy.

Implications: These results from the first hemophilia B gene therapy study in patients demonstrate the potential safety and efficacy for an adeno-associated viral vector gene therapy for hemophilia. Moreover, the approach may be useful in other genetic disorders.

Kay MA, Manno CS, Ragni MV, et al: Evidence for gene transfer and expression of factor IX in haemophilia B patients treated with an AAV vector. Nature Genetics, 24(3):257-61. 2000.

Gene-Environment Interactions – A New Clue with Implications for Asthma

Background: Most diseases arise from the complex interaction of underlying genetic susceptibility and environmental exposures that occur over time. Although most people think of environmental exposures in terms of synthetic chemicals, some of our most important exposures are from the natural world. These exposures would include the nutrients in our food, the pathogens normally found in soil and air, and the by-products produced by insects and mites in our homes.

One such exposure is endotoxin. Endotoxin is produced by molds and is commonly found in house and grain dusts. Inhalation of endotoxin has been implicated in asthma and other respiratory diseases and has been shown to cause chest tightness, wheezing, and difficulty in breathing. Endotoxin-induced asthma is a particular problem in farming occupations that involve handling grain and subsequent exposure to grain dusts. Researchers have recently discovered an important genetic component underlying susceptibility to endotoxin that has implications not only for asthma, but potentially for other airway diseases such as acute lung injury, cystic fibrosis, and pneumonia.

Advance: These investigators studied the response of 83 healthy human participants to increasing concentrations of endotoxin equal to the amount a grain handler or farmer would be exposed to during a normal work-day. The results demonstrated differences in the constriction of the airways between the participants – some exhibited normal constriction while others were affected to a much lesser degree. Further genetic testing led the investigators to attribute the difference in response to mutations in the toll-like receptor- 4 (TLR4) gene. These findings were further substantiated by *in vitro* experiments conducted on cell cultures obtained from people with the mutation. By adding a normal copy of the TLR4 gene to the cells in culture, the normal cell response to endotoxin was recovered.

Implication: These findings may have future implications on the treatment of asthma and other respiratory diseases. Scientists may be able to capitalize on these findings by designing drugs that will bind to normal TLR4 receptors and thus prevent the bronchoconstriction seen in asthmatics. However, the researchers warn that people with the TLR4 mutation may be more susceptible to blood-borne infections because they may not respond as readily to early signs of infection.

Arbour NC, Lorenz E, Schutte BC, et al: TLR4 mutations are associated with endotoxin hyporesponsiveness in humans. Nature Genetics, 25(2):187-91. 2000.

Overcoming Obstacles to Islet Transplantation for Type 1 Diabetes

Background: Of the 16 million people in the U.S. with diabetes, approximately 800,000 people have type 1 diabetes. This is an autoimmune disease in which the body's immune system attacks and destroys its own insulin-producing beta cells in the islets of the pancreas. Once the islets have been destroyed, patients need insulin injections to live. Researchers have been pursuing means to restore insulin-producing capacity through transplantation of the whole pancreas or of islets isolated from donor pancreata. Despite the well-publicized recent success of islet transplantation in Canada, several obstacles remain to the widespread application of this technique. These include the difficulty of obtaining adequate numbers of islets for transplantation and the requirement for lifelong immunosuppressive therapy. Current immunosuppression can be toxic to transplanted islets and can cause an increased susceptibility to infections and malignancies.

Advance: Recently, attention has focused on the possible use of controlled differentiation of stem cells to obtain specialized cells useful in treating many diseases. NIH-supported investigators have generated islets in the laboratory using cells isolated from the pancreatic ducts from the non-obese diabetic mice, before they developed diabetes. These duct cells produced islets throughout a long-term culture period. When these islets were implanted into the kidney of non-obese diabetic mice, the mice were able to be weaned off insulin injections and exhibited a reduction in blood glucose levels, which approached normal glucose levels. Future experiments will be necessary to verify that the reduction in glucose levels seen in these experiments was the result of the islets implanted into the kidney.

Other NIH-supported investigators have undertaken efforts to cultivate human pancreatic ductal cells in the laboratory. These investigators showed that pancreatic tissue normally discarded after isolation of islets could be cultured to produce islet-like clusters of pancreatic endocrine cells. These clusters were capable of producing insulin in response to glucose in culture. This approach suggests that meaningful amounts of new human islet tissue can be obtained from duct cells.

Another group of NIH-supported investigators has recently published data on successful islet transplantation without the need for long-term immunosuppression. This research has resulted in improved metabolic function in non-human primates. They transplanted islets isolated from the pancreas of non-diabetic monkeys into the portal vein of diabetic monkeys. They administered an immunotoxin to the animals for four days following surgery. After surgical recovery, the animals underwent metabolic testing. The results demonstrated that control of blood glucose had been achieved: none of the animals required insulin injections nine days post-transplantation. Tolerance induction – teaching the immune system to accept foreign tissue as "self" – alleviated the need for long-term immunosuppression. Hence, after this short-term immunotoxin treatment, the monkeys did not experience any apparent immunological compromise, such as infections, malignancies, or development of autoimmunity.
Implications: For people with type 1 diabetes, insulin is a therapy, not a cure, and it does not provide protection from the severe long-term complications of the disease. The availability of a sufficient

number of islet cells for transplantation and the means to prevent their rejection without long-term immunosuppressive treatment are prerequisites to making islet transplantation a reality for the 800,000 people currently diagnosed with type 1 diabetes. Advances such as those described above represent major steps toward achieving the reality of a "cure" for type 1 diabetes.

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Bonner-Weir S, Taneja M, Weir GC, Tatarkiewicz K, Song KH, Sharma A, and O'Neill JJ: In vitro cultivation of human islets from expanded ducal tissue. Proceedings of National Academy of Sciences, 97(14):7999-8004. 2000.

Contreras JL, Eckhoff DE, Cartner S, et al: Long-term functional islet mass and metabolic function after xenoislet transplantation in primates. Transplantation, 69(2):195-201. 2000.

Novel Approaches in Gene Transfer Delivery Methods

Background: Gene transfer is a novel approach to alter the expression of a person's genes in an effort to treat, cure, or ultimately prevent disease. One of the greatest challenges in gene transfer is to develop ways to deliver therapeutic materials to the cells of a patient in a way that is specific, efficient and safe. Many viral-based vectors have shown potential as vehicles for gene delivery including: adenovirus, herpesvirus, retrovirus, and adeno-associated virus. However, there are some drawbacks in the use of viral vectors because the viral proteins can induce an immune response. A major shortcoming of the use of AAV vectors is the size limitation of the genetic material that can be incorporated into the vector. The packaging size of AAV vectors is less than 5,000 bases (5 kb). Thus, diseases caused by defective genes with coding regions larger than 5 kb, such as Hemophilia A or Duchenne muscular dystrophy, cannot be treated using the AAV vector systems. NIH-supported researchers are working to improve existing methods for delivery of therapeutic genes, as well as to develop new methods.

Advances: NIH-supported researchers have demonstrated that recombinant AAV (rAAV) vectors can indeed be used to deliver therapeutic genes larger than 5 kb. Three groups have independently developed methods to split the coding region into two rAAV vectors that are reassembled in the cell. It has been shown that infection of muscle tissue with rAAV vectors involved the conversion of the single-stranded viral DNA to circular intermediate forms in head-to-tail concatemers that correlate with long-term gene expression. Investigators have shown that functional expression of erythropoietin – a protein involved in the formation of red blood cells – can be achieved after co-infection of fibroblasts and muscles with two rAAV vectors encoding separate regions of the human erythropoietin gene. These two separate, smaller regions will "splice" together in the cell to result in the complete gene sequence needed to produce the functional protein. In addition, they have demonstrated that levels of erythropoietin resulting from co-infection of the two rAAV vectors, each containing a distinct portion of the gene, are capable of protecting a mouse model from anemia induced by renal failure.

Another group of NIH-supported researchers is examining alternative approaches to the use of viral vectors for gene therapy. One method currently under study is the use of "naked DNA," either alone or in conjunction with other materials. In the past decade, this approach has been successfully used *in vivo* and has produced transient gene expression in the liver, lung, muscle, skin, and heart. One approach to increase the integration of nonviral vectors in animals to prolong gene expression is to incorporate elements of a DNA transposon into the vector. Transposons are naturally occurring genetic elements capable of moving from one chromosomal location to another, often by a "cut and paste" mechanism. The specific transposon, *Sleeping Beauty*, has previously been shown to have the ability to insert foreign genes into the chromosomes of cultured vertebrate cell lines, including mouse embryonic stem (ES) cells and human cells. Investigators have demonstrated that *Sleeping Beauty* can facilitate integration of naked DNA into mouse chromosomes, thereby resulting in long-term therapeutic gene expression in normal and hemophilic mice. This method resulted in partial correction of bleeding in the

mouse model of hemophilia B. This is the first demonstration of transposition-mediated gene transfer in an adult animal.

Implications: The finding that two independent vectors, encoding large genes in two segments, can be used to deliver therapeutic proteins will broaden the use of rAAV vectors to diseases in which the defective gene was previously thought to be too large for effective gene transfer techniques. This finding will thus significantly increase the utility of the rAAV vector for gene therapy of inherited and acquired diseases. In related research, the use of transposons as an alternative to viral vectors represents a major advance in the development of stable nonviral gene transfer systems. Furthermore, the transposons may permit lifelong gene expression with a single administration of the vector. These plasmid-based vectors are amenable to large-scale manufacture and could prove beneficial in human gene therapy applications. However, before this technology is widely used, more research must be done on the immunological response to long-term exposure to these vectors.

Duan D, Yue Y, Yan Z, Engelhardt JF: A new dual-vector approach to enhance recombinant adeno-associated virus-mediated gene expression through intermolecular cis activation. Nature Medicine, 6(5):595-8. 2000. Discussion: Nature Biotechnology, 18:497-8. 2000.

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Sun L, Li J, Xiao X: Overcoming adeno-associated virus vector size limitation through viral DNA heterodimerization. Nature Medicine, 6(5):599-602. 2000.

Nakai H, Storm TA, Kay MA: Increasing the size of rAAV-mediated expression cassettes in vivo by intermolecular joining of two complementary vectors. Nature Biotechnology, 18(5):527-32. 2000.

Yant SR, Meuse L, Chiu W, Ivics Z, Izsvak Z, Kay MA: Somatic integration and long-term transgene expression in normal and haemophilic mice using a DNA transposon system. Nature Genetics, 25(1):35-41. 2000.

Mutant Mice Offer Clues to Calcium Cycling in the Heart

Background: Dilated cardiomyopathy, a condition marked by reduced contractility of heart muscle, is a major form of heart failure. The heart's pumping action at the cellular level is controlled in part by the cyclical movement of calcium ions into and out of a membrane sac – the sarcoplasmic reticulum – in heart muscle cells. But the enzymes and biochemical events that regulate calcium cycling are poorly understood. Because defective calcium cycling lies at the root of many heart disorders, deeper knowledge of these processes could lead to novel strategies for treating and preventing progressive dilated cardiomyopathy, as well as other heart conditions.

Advance: By studying transgenic mice that have targeted mutations in muscle-specific enzymes that affect calcium transport, investigators have demonstrated that the progressive defect in dilated cardiomyopathy is linked to these key molecules. Results from these investigations showed that enhanced inhibition of these enzymes alters calcium reuptake. The investigators further demonstrated that both inhibitory molecules and enzyme/inhibitor-complexes are involved and can prevent heart failure.

Implication: Heart failure is the leading cause of combined morbidity and mortality in the United States and other developed nations. The characterization of important rate-limiting steps in the calcium-reuptake metabolism of heart muscle cells may lead to development of new designer drugs or gene therapies to successfully prevent the sickness and death caused by dilated cardiomyopathy and end-stage heart failure.

Minamisawa S, Hoshijima M, Chu G, et al: Chronic phospholamban-sarcoplasmic reticulum calcium ATPase interaction is the critical calcium cycling defect in dilated cardiomyopathy. Cell, 99(3):313-22. 1999.

New Vector Enhances Gene Transfer

Background: The blood-forming, or hematopoietic, stem cells found in bone marrow are attractive targets for gene therapy. By inserting therapeutic genes into these cells, which give rise to many different types of blood cells, gene therapists hope to establish a long-term source of genetically altered cells in the bone marrow. Ultimately, genetic manipulation of hematopoietic stem cells (HSCs) may prove effective in treating a variety of genetic, infectious, cancer-causing, and other diseases. But once administered to living organisms, HSCs and their cellular offspring often rapidly lose their ability to express therapeutic genes. This problem might be solved with an improved "vehicle" for delivering foreign genes to the HSCs.

Advance: Investigators demonstrated that a recombinant simian papovavirus (SV40) can effectively deliver foreign genes to both human and simian HSCs in culture. When transplanted into mice, the HSCs continued to express their foreign (or therapeutic) genes for at least three months, which is a remarkable achievement. These highly efficient vectors transported genes into almost all targeted cells, whether resting or dividing. The researchers further observed that SV40 vectors can deliver foreign genes to nondividing cell populations, such as neurons, and can also deliver genes at high efficiency to the liver.

Implications: Among the obstacles to effective gene therapy are major difficulties in producing vectors, or "vehicles," that can effectively and efficiently deliver therapeutic genes to cells. SV40-derived vectors may provide such a vehicle, especially to hematopoietic stem cells that can differentiate into a wide variety of tissues. Such a vector, if proven safe as well as effective, could lead to the treatment and potential cure of significant numbers of genetic and infectious diseases.

Strayer DS, Pomerantz RJ, Yu M, et al: Efficient gene transfer to hematopoietic progenitor cells using SV40-derived vectors. Gene Therapy, 7(10):886-95. 2000.

Dopamine Receptor Agonists and Parkinson's Disease Treatment in Non-human Primate Models

Background: Parkinson's disease (PD) affects primarily the elderly. The degenerative process can be defined by stages. At the onset of clinical symptoms, unilateral tremor, limb stiffness, slowness of movement, and gait disturbances appear but do not interfere with daily life. Intermediate stages, bilateral tremors appear and disabilities begin to interfere with daily activities. Advanced stages are characterized by sufficient disability to require living assistance. Current and future strategies for treating patients with PD use therapeutic approaches appropriate to the degree of functional impairment. At present, drug therapies are largely designed to replace dopamine. Currently, at least five distinct dopamine receptor subtypes have been identified. Most treatment drugs are targeted for the D2 - like subfamily of receptors. However, D1 - receptors have not been systematically examined for their therapeutic potential.

Advance: Selective D1 dopamine receptor agonists exert antiparkinsonism effects in the 1–methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) monkey model of PD and in human PD. A Parkinsonism rating scale has been developed in MPTP-treated monkeys to assess Parkinsonism signs and effects of drugs. Mild and advanced stages of PD were produced in monkeys. Based on the therapeutic efficacy and side effect profiles derived from studying these models, D1 agonists appear to be more promising for the treatment of advanced than of mild stages of PD.

Implications: Drugs targeted to specific receptor subtypes may offer treatment advantages in terms of efficacy, tolerance and potential side effects. Having an animal model of PD that corresponds to the early and late stages of human PD will enable the evaluation of D1 agonists and to assess their therapeutic potential for treating mild or advance PD in humans. It appears that D1 agonist may be more appropriately prescribed for end stage treatment of PD, a time when L-dopa (D2) efficacy wanes and dyskinesias are frequently encountered in patients. The ability to identify and assess efficacy in an animal model will clearly expedite the development of effective therapeutics.

Goulet M, Madras BK: D_1. Dopamine receptors agonist are more effective in alleviating advanced than mild Parkinsonism in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated monkeys. Journal of Pharmacology and Experimental Therapeutics, 292(2):714-24. 2000.

Experimental Technique Shows Promise in Treating Liver Metastases in Patients with Colorectal Cancer

Background: An estimated 130,000 people in the United States will learn this year that they have colorectal cancer. Excluding skin cancer, colorectal cancer is the third most commonly diagnosed cancer for both men and women, and the second leading cause of cancer-related death. In 60% of patients with colorectal cancer, the tumor spreads to the liver. More than 14,000 patients undergo surgery each year to remove these liver metastases. However, following surgery, there is currently no standard way of treating remaining microscopic tumors (micrometastases) or preventing tumors from spreading to other organs. Additionally, some patients have liver metastases that – because of their location, number, or other factors – cannot be surgically removed. Despite aggressive chemotherapy, life expectancy for patients with inoperable liver metastases of colorectal cancer ranges from 2 months to 2 years.

Advance: Hepatic arterial infusion (HAI) is an experimental technique for delivering high doses of chemotherapeutic drugs directly to liver tumors while minimizing toxicity to the liver and other organs. Unlike other organs, the liver has a dual blood supply; liver tumors get their blood supply largely from the hepatic artery, whereas normal liver cells obtain theirs from the portal vein. HAI works by infusing drugs through a catheter or pump in the hepatic artery. The technique has recently shown promising results in clinical trials conducted by two groups of NIH-supported investigators. One trial compared the efficacy of a combination of standard chemotherapy and HAI with standard chemotherapy alone in 156 patients who had undergone surgical removal of liver metastases of colorectal cancer. After 2 years, overall survival was 86% in the group treated with combined therapy and 72% in the group receiving standard chemotherapy alone; survival free of recurrence of liver tumors was 90% and 60%, respectively. In the second trial, 34 patients with inoperable primary or metastatic liver tumors were treated with a variant of HAI known as isolated hepatic perfusion. Among patients with colorectal cancer, the response rate was 80 to 90%; the median duration of response was over 18 months. In most patients, treatment resulted in significant regression of bulky liver tumors.

Implications: These studies show that patients with both operable and inoperable liver metastases of colorectal cancer can benefit from HAI. The larger of the two studies showed that HAI plus standard chemotherapy not only decreased the rate of liver tumor recurrence but also improved 2-year overall survival compared with standard chemotherapy alone. (Previous studies had failed to make clear whether HAI actually extended patients' lives.) The results of the second study suggest that with continued refinement and more widespread application, isolated hepatic perfusion may be considered a suitable treatment alternative for patients with inoperable liver tumors.

Kemeny N, Huang Y, Cohen AM: Hepatic arterial infusion of chemotherapy after resection of hepatic metastases from colorectal cancer. The New England Journal of Medicine, 341(27):2039-48. 1999.

Novel Bone Marrow Transplant Procedure Offers New Hope to Patients With Cancers of the Blood

Background: Bone marrow transplants are an effective treatment for patients with cancers of the blood such as leukemia, multiple myeloma, and non-Hodgkin's lymphoma. The bone marrow is where most hematopoietic stem cells – immature cells from which all blood cells develop – are found. A bone marrow treatment involves destroying the patient's diseased bone marrow with high doses of chemotherapy or radiation, and replacing it with healthy marrow from a matched donor. High doses of chemotherapy or radiation are necessary to eliminate the underlying disease and to overcome two biological barriers to donor transplantation: host-versus-graft (HVG) reaction, or rejection of the transplanted tissue by the patient's immune system, and graft-versus-host disease (GVHD), in which the transplanted tissue mounts an immune attack on the patient's cells. Conventional bone marrow transplants are not normally performed on patients older than 55 or on patients who are unfit to withstand the intensive, highly toxic treatment regimen. Thus, this potentially curable treatment is currently available only to the minority of patients with cancers of the blood who are under 55 and in good physical condition.

Advance: NIH-supported researchers have developed an experimental bone marrow transplant procedure known as a "mini-transplant." Instead of using high doses of radiation to kill cancer cells, this procedure takes advantage of the immune properties of donor stem cells. In a process called the "graft versus tumor" effect, the donor cells recognize and kill cancer cells while tolerating the patient's normal cells and tissues. The mini-transplant uses a much lower dose of radiation than a conventional bone marrow transplant – just enough to suppress the patient's immune system without wiping out the bone marrow. The patient also receives drugs to control HVG reaction and GVHD. The procedure has been performed on over 80 patients whose average age was 56. Patients did not experience hair loss, severe mouth sores, low levels of blood cells, and other adverse effects that frequently occur in patients receiving conventional transplants. Five patients (6%) died within the first year; this compares with a mortality rate of 25% for younger patients with chronic myeloid leukemia who underwent conventional transplants. Most mini-transplant patients went home from the hospital the same day. By contrast, recipients of conventional transplants are hospitalized in intensive care for 2-3 months. Rates of GVHD were similar to those that occur after conventional transplants.

Implications: These study results challenge the notion that more intensive treatment is better and help to delineate the role of the immune system in cancer progression and treatment. If shown to be effective in phase III clinical trials, the mini-transplant procedure will make bone marrow transplants more available, less toxic, less costly, and more effective. Furthermore, the procedure may be effective in treating solid tumors as well as autoimmune diseases, genetic diseases, and sickle cell anemia.

Xun CQ, McSweeney PA, Boeckh M, Storb RF, Broudy VC, Thompson JA: Successful nomyeloablative allogeneic hematopoietic stem cell transplant in an acute leukemia patient with chemotherapy-induced marrow aplasia and progressive pulmonary aspergillosis. Blood, 94(9):3273-6. 1999.

Early Hormonal Therapy Extends Lives of Prostate Cancer Patients, Study Shows

Background: Prostate cancer is the most commonly diagnosed form of cancer, other than skin cancer, among men in the United States, and is second only to lung cancer as a cause of cancer-related death among men. This cancer is most common among men 65 and older. A sharp increase in the number of cases of prostate cancer diagnosed annually in recent years is believed to be largely due to more widespread screening for the disease, particularly by means of the prostate-specific antigen (PSA) test. Treatment for prostate cancer depends on the stage of disease and on the patient's age and overall health; options include surgery, radiation, and drug treatment. Because male hormones (especially testosterone) can help prostate cancer to grow, one treatment approach (known as hormonal therapy) is to use drugs or surgery to reduce levels of male hormones. Use of this therapy has generally been confined to patients with more advanced disease. Side effects of hormonal therapy can include hot flashes, impaired sexual function, and loss of desire for sex. A longstanding and controversial question in prostate cancer treatment has been whether earlier use of hormonal therapy can prolong survival for patients who have not been cured by surgery or radiation.

Advance: NIH-supported investigators conducted a randomized clinical trial in which men who had had surgery for early-stage prostate cancer were assigned either to receive immediate hormonal therapy or to simply be observed until their disease progressed. All of the men were node positive – they had microscopic tumor metastases in their lymph nodes – putting them at high risk for recurrence of their cancer. A total of 98 patients were enrolled in the study between 1988 and 1993 and followed for 7 years on average. At the end of the follow-up period, 7 of 47 men who received immediate hormonal therapy had died, as compared with 18 of 51 men in the observation group. Three of the deaths in the treatment group and 16 of the deaths in the observation group were due to prostate cancer. At the time of the last follow-up, 36 men in the treatment group (77%) and 9 men in the observation group (18%) were alive and had no evidence of recurrent disease.

Implications: The results of this trial, together with evidence from previous studies, support the hypothesis that early hormonal therapy may prolong the survival of men with prostate cancer. The trial has changed the standard of care for node-positive prostate cancer patients, and the dramatic results also suggest that early administration of hormonal therapy could extend the lives of many other prostate cancer patients.

Messing E, Manola J, Sarosdy M, Wilding G, Crawford ED, Trump D: Immediate hormonal therapy compared with observation after radical prostatectomy and pelvic lymphadenectomy in men with node-positive prostate cancer. The New England Journal of Medicine, 341(24):1781-8. 1999.

New Approaches to Bone Cancer Pain

Background: The most common symptom of bone cancer is pain. Pain occurs in primary bone cancer and in cancer that has spread from breast, prostate, ovary, lung, or other sites. The pain is dull, constant, and increases in intensity over time, with intermittent episodes of extreme pain that may be spontaneous or provoked by movement. The best available pain treatments are often inadequate to treat bone pain, or are fraught with troublesome side effects. Poor understanding of what causes bone pain seriously hampers the development of better treatments.

Advance: Scientists have developed a new approach to bone pain by trying to understand what causes the problem. Tumors in bone induce an imbalance between the normal ongoing processes of bone formation and bone resorption, resulting in net bone destruction that often accompanies cancerous bone pain. Researchers reasoned that this bone destruction may cause bone pain in several ways. Osteoclasts, the cells responsible for resorbing bone, maintain an acidic microenvironment, and sensory nerves that innervate bone are very sensitive to acidity. These nerves are also sensitive to chemical factors that bone resorption releases. Furthermore, weakened bone may fracture and mechanically stimulate pain fibers. Finally, nerves relay pain signals through the spinal cord to the brain. Changes in the spinal cord underlie increasing pain sensitivity (sensitization) in many chronic pain conditions.

To test these ideas scientists first developed a mouse model of bone cancer, by injecting malignant bone cancer cells into mouse leg bones. The tumor cells provoked bone destruction similar to what happens in human bone cancer, and mice showed pain behaviors that seemed to reflect the human experience. Changes in the spinal cord of the mice were similar to those seen during pain sensitization in other chronic pain states.

With a good animal model established, researchers then administered osteoprotegrin (OPG) to the bone cancer mice. OPG is a natural chemical signal that blocks the bone resorbing activity of osteoblasts. OPG not only blocked the bone destruction, but also substantially diminished pain-related behaviors, and prevented the reorganization of the spinal cord that reflects pain sensitization. No serious side effects were apparent in these brief studies, which treated mice for only 17 days.

Implications: If OPG can block tumor induced bone destruction, bone pain, and spinal cord sensitization in people, safely and without serious side effects, it would enhance the quality of life for many people with bone cancer. If OPG itself is not a suitable drug, scientists may be able to develop agents that mimic its beneficial effects. More generally, chronic pain from cancer and many other conditions seriously affects the quality of life for many people. These experiments reflect a trend in the development of pain treatments that relies upon a mechanistic understanding of the neurobiological basis of pain states, rather than the usual empirical screens for drug candidates. With the rapidly increasing understanding of the neuroscience of pain this approach is becoming increasingly viable.

Honore[1] P, Luger[1] PNM, Sabino[1] MAC, et al: Osteoprotegerin blocks bone cancer-induced skeletal destruction, skeletal pain and pain-related neurochemical reorganization of the spinal cord. Nature Medicine, 6(5):521-8. 2000.

Progress in Enzyme Replacement Therapy for Fabry Disease

Background: Fabry disease is the second most prevalent hereditary metabolic storage disease of humans. Symptoms typically first appear during childhood or adolescence with recurrent episodes of severe pain in the extremities, characteristic skin lesions called angiokeratomas, and effects on the cornea. With increasing age the disease affects vital organs. Death usually occurs during the fourth or fifth decade from effects on the kidneys, heart, or blood vessels of the brain. The disease is X-linked recessive, so most people affected are males. Females who carry the gene may suffer the full blown clinical syndrome or have milder symptoms and a longer life than males. Fabry disease is caused by insufficient activity of the enzyme α-galactosidase A that degrades a lipid (fatty substance) called globotriaosylceramide. This substance accumulates throughout the body, causing damage especially to blood vessels in the brain, heart and kidneys and to the kidney tubules.

Advance: NIH intramural investigators previously demonstrated that intravenous administration of the enzyme a-galactosidase A temporarily reduces the levels of globotriaosylceramide in the blood of Fabry patients. The enzyme was laboriously isolated from placental tissue, and lack of sufficient quantities hampered further tests. To overcome this limitation scientists prepared the enzyme using recombinant DNA technology and human cells in culture. With adequate supplies of enzyme in hand, researches conducted a phase I safety and dose-escalation clinical trial of this enzyme in patients with Fabry disease and demonstrated that the procedure was safe and that it caused a reduction of globotriaosylceramide in the liver, blood, and urine. The latter observation was particularly gratifying because it indicated possible improvement in impaired kidney function that is characteristic of this disorder. Moreover several of the patients were able to permanently discontinue the medications they were taking for the pains in their hands and feet.

Implications: The results of this trial provided the basis for a double-blind placebo-controlled phase II clinical efficacy trial of enzyme replacement therapy in Fabry disease that has recently been completed. The reduction of pain in the hands and feet was confirmed. Tests also proved that kidney and heart function improved. These findings are being prepared for publication and formed the basis of a Biologics License Application that was submitted to the U.S. Food and Drug Administration in June 2000 for the approval of this enzyme for treatment of patients with Fabry disease.

Schiffmann R, Murray GJ, Treco D, et al: Infusion of α-galactosidase A reduces tissue globotriaosylceramide storage in patients with Fabry disease. Proceedings of the National Academy of Sciences, 97(1):365-70. 2000.

Gene Therapy Strategy for Parkinson's Disease

Background: Parkinson's disease disrupts movement control circuits of the brain. The disease usually progresses slowly, reflecting the death of nerve cells in a brain region called the substantia nigra that produce the neurotransmitter dopamine. The drug levodopa can mask symptoms by boosting the dopamine output of the remaining subtantia nigra dopamine nerve cells. Ultimately this therapy fails when too many of these cells die and side effects of high doses can be severe.

Several years ago scientists isolated a neurotrophic factor called GDNF (glial cell line derived neurotrophic factor). Neurotrophic factors are natural chemicals, usually small proteins, that promote survival and growth of neural cells, and GDNF is especially potent for dopamine neurons. Short term experiments in animal models of Parkinson's disease have reinforced the hope that GDNF might help forstall disease progression, but this goal has been thwarted by the difficulties in providing sustained delivery of GDNF to appropriate cells within the human brain. The blood brain barrier and the large size of the human brain, compared with animals, are among the obstacles to adequate delivery.

Advance: Gene therapy is a potentially powerful method for delivering neurotrophic factors to the brain. Through genetic engineering technology, a team of scientists adapted a type of virus, called a lentivirus, to infect neural cells. The virus carries into the cells the gene for GDNF and control signals to trigger cells to produce this molecule. To test this therapeutic strategy they used two different non-human primate models of Parkinson's disease, a aged-monkey model and a young monkey model in which dopamine cells have been damaged by the toxin MPTP. Several types of structural and biochemical measurements demonstrated that this procedure reduced the degeneration of dopamine cells, and behavioral tests confirmed the benefits on movement control.

Implications: These results indicate that a gene therapy strategy using a lentiviral delivery system to produce GDNF may be a viable approach to slow the progression of Parkinson's disease. Early Phase I safety trials are in the planning stages. From a broader perspective, this advance highlights the potential of gene therapy even for neurological diseases, like most Parkinson's, that are not caused by defective genes.

Kordower JH, Emborg ME, Bloch J, et al: Neurodegeneration prevented by lentiviral vector delivery of GDNF in primate models of Parkinson’s disease. Science, 290(5492):767-73. 2000.

Embryonic Stem Cells Help Recovery from Spinal Cord Injury in Rats

Background: In the last few years several promising treatment strategies for spinal cord injury have begun to emerge from laboratory studies in animals. These include approaches to minimize the "secondary damage" that continues in the hours and days following the initial trauma by blocking "excitotoxicty" or interupting "cell suicide," as well as attempts to encourage regeneration by supplying growth factors, neutralizing barriers to regeneration, surgically bridging gaps, and transplanting fetal tissue or other cells. Virtually all of these success stories in animal models were applied quickly following initial trauma. For the hundreds of thousands of people around the world with chronic disability from spinal cord injury we need treatments that can encourage recovery of function after the injury process is complete.

Embryonic stem (ES) cells are unspecialized cells in the early embryo that multiply and give rise to all of the specialized cell types of the body. ES cell therapies have been proposed for many different nervous system disorders because these cells have so many potentially useful capabilities. For spinal cord injury transplanted ES cells might give rise to new nerve cells that carry signals between the brain and the body; form new oligodendrocytes that ensheath nerve fibers with insulation (myelin) essential to proper electrical conduction of signals; encourage regeneration by filling in the physical gap that forms in the spinal cord, thus eliminating a physical barrier to growing nerve fibers; or release substances that prevent cells from dying or encourage them to grow.

Advance: A research team has now developed an ES cell transplant method that improves the outcome from spinal cord injury in rats even when the treatment is delayed until nine days following the injury. The scientists used mouse ES cells that had been treated with retinoic acid, a natural signal from the developing nervous system that directs ES cells to specialize to form neural tissue. Using various labels that identify different cell types, the researchers found that some of the transplanted cells survived for at least five weeks and gave rise to new neurons, oligodendrocytes, and astrocytes, another type of supporting cell. In behavioral tests, the hind limbs of the treated rats (but not controls) regained some coordinated movement and could partially support the body's weight, although the animals could not walk normally.

Implications: Even a small improvement of functional connections in the spinal cord can make an important difference in behavioral abilities, so these results are certainly encouraging, especially with the recent isolation and purification of human ES cells. However, much more work in animals is needed before the procedure is ready for people with spinal cord injury. The scientists must determine why relatively few transplanted cells survive, determine how the stem cells are helping, and optimize the procedure. All of this depends on continued progress in understanding the biology of stem cells. Finally, extensive work is necessary to extend the procedure beyond nine days post-injury and to establish its long term safety.

McDonald JW, Liu XZ, Qu Y, et al: Transplanted embryonic stem cells survive, differentiate and promote recovery in injured rat spinal cord. Nature Medicine, 5(12):1410-2. 1999.

New Drug is Safe and Effective for Children and Teenagers with Juvenile Rheumatoid Arthritis

Background: Enbrel (etanercept) belongs to a new class of drug treatments called biological agents, which are designed to interfere with the specific biological process of a disease. Enbrel is a tumor necrosis factor (TNF) antagonist, a substance that blocks the action of TNF, a naturally occurring protein in the body that helps cause inflammation. Enbrel has been shown to be effective in treating adults with rheumatoid arthritis, and researchers have considered the safety and efficacy in children with the juvenile form of rheumatoid arthritis..

Advance: Researchers conducted a clinical trial of Enbrel in children ages 4 to 17. In 3 months, 74 percent responded with measurable improvement. After 1 year of treatment, 80 percent of the patients were improved, 76 percent no longer had morning stiffness, and 32 percent no longer had any joint pain.

Implications: Juvenile rheumatoid arthritis (JRA) is a type of arthritis that causes joint inflammation and stiffness for more than 6 weeks in a child 16 years of age or younger. Before Enbrel, many children with severe JRA had a poor response to existing treatment options and had to stop attending school. This new drug offers the hope that children with JRA can lead more normal lives.

Lovell DJ, Giannini EH, Reiff A, et al: Etanercept in children with polyarticular juvenile rheumatoid arthritis. The New England Journal of Medicine, 342(11):763-9. 2000.

Analysis Shows Glucosamine/Chondroitin Sulfate is Probably Useful for Osteoarthritis

Background: Glucosamine and chondroitin sulfate have received significant media attention for the treatment of osteoarthritis (OA) and have been used in Europe for over 10 years. Physicians in the United States and the United Kingdom have been skeptical about these products because of concerns about the quality of scientific trials conducted to test them. Glucosamine and chondroitin sulfate, sold in the United States as dietary supplements, are natural substances found in and around the cells of cartilage. Researchers believe these substances may help in the repair and maintenance of cartilage.

Advance: Researchers conducted a systematic analysis of clinical trials on glucosamine and chondroitin sulfate for treating OA. They located 37 studies of the compounds in OA treatment by a thorough review of the literature going back more than three decades. Of these, 15 trials published between 1980 and 1998 met their criteria: double-blind, randomized placebo-controlled trials that lasted 4 or more weeks, tested glucosamine or chondroitin for OA of the knee or hip, and reported data that the team could extract of the effect on treatment of OA symptoms. Six of the 15 trials involved glucosamine and 9 used chondroitin sulfate.

The results of the analysis indicated that glucosamine and chondroitin sulfate may have some efficacy against the symptoms of OA. Based on data from the trials, the researchers calculated an overall "effect size" for the two compounds: the figure 0.2 is considered a small effect; 0.5, moderate; and 0.8, large. They calculated an effect size for glucosamine of 0.44 and for chondroitin sulfate of 0.78, but reported that these values "were diminished when only high-quality or large trials were considered." The investigators recommend that additional, rigorous, independent studies be done of these compounds to determine their true efficacy and usefulness.

Implications: About 21 million adults in the United States have OA, a degenerative joint disease caused by breakdown of cartilage, which cushions the end of bones within the joint. NIH has awarded a 4-year research contract to conduct the first U.S. multicenter study to investigate glucosamine and chondroitin sulfate for OA of the knee. The meta-analysis cited in this advance adds to the scientific foundation for the newly launched multicenter study of these compounds.

McAlindon TM, LaValley MP, Gulin JP, Felson DT: Glucosamine and chondroitin sulfate for treatment of osteoarthritis: A systematic quality assessment and meta-analysis. Journal of the American Medical Association, 283(11):1469-75. 2000.

Osteoporosis Therapies Prevent Bone Cell Death

Background: Drugs in the bisphosphonate family have brought about a marked improvement in the clinical management of diseases that involve bone loss. Bisphosphonates block the action of osteoclasts, cells that resorb (dissolve) bone, but it is not clear how these drugs work.

In the last 2 years, evidence has accumulated indicating that bone loss is often associated with the death of osteoblasts, cells that form bone. The cell death observed is of a particular type, called programmed cell death, or apoptosis, in which the cell dies in response to external signals. Other bone cells, called osteocytes, which are derived from osteoblasts when the bone-forming cells become embedded in the bone they have formed, also undergo apoptosis under conditions that lead to bone loss.

Advance: In 1998, researchers showed that certain steroid drugs induce the death of osteoblasts and osteocytes. In their most recent work, these researchers demonstrated that bisphosphonates can prevent steroid-induced apoptosis of osteoblasts and osteocytes. They also showed that bisphosphonates induce certain biochemical changes in the cells that are known to block apoptosis. Finally, they showed that calcitonin, a naturally occurring protein that, like bisphosphonates, can prevent bone loss, also prevents apoptosis of osteoblasts and osteocytes. These findings suggest that the effectiveness of bisphosphonates and calcitonin is due not just to the inhibition of bone resorption but to preservation of osteocytes and osteoblasts as well.

Implications: Although antiresorptive drugs like bisphosphonates and calcitonin can prevent bone loss, no currently available therapy can restore bone that has already been lost. If the mechanism by which bisphosphonates and calcitonin prevent apoptosis in osteoblasts and osteocytes can be identified, it may be possible to design other therapies that exploit this effect to an even greater degree, leading to therapies that actually increase bone mass.

Plotkin LI, Weinstein RS, Parfitt AM, et al: Prevention of osteocyte and osteoblast apoptosis by bisphosphonates and calcitonin. The Journal of Clinical Investigation, 104(10):1363-74. 1999.

Gene Therapy Restores Full Function in a Limb-Girdle Muscular Dystrophy

Background: Limb-girdle muscular dystrophies (LGMD) are caused by genetic mutations that disrupt a critical molecular complex on muscle membranes. This sarcoglycan complex, which consists of four subunits, helps protect the muscle membrane from disruption during forceful contractions, and it links components of the extracellular matrix to the inside of the muscle cell.

Advance: Using hamsters that had a naturally occurring form of LGMD caused by a defective sarcoglycan subunit, researchers restored normal physiological function to the muscle with gene therapy. They delivered a normal subunit gene by means of a vector, enabling new genetic material to enter the cell and allowing the muscle to develop to normal size and weight with no pathological muscle hypertrophy.

Implications: The restoration of full muscle force recovery and reversal of pathological hypertrophy after injection of a vector to correct a defect causing a form of LGMD demonstrates the feasibility of in vivo gene therapy for LGMD and provides a strong rationale for the development of gene therapy for other muscular dystrophies.

Xiao X, Li J, Tsao YP, et al: Full functional rescue of a comple muscle (TA) in dystrophic hamsters by adeno-associated virus vector-directed gene therapy. Journal of Virology, 74(3):1436-42. 2000.

Oxygen Has a Positive Effect on Wound Healing

Background: The healing of chronic wounds is a major health problem in the United States, especially among the elderly and the very young with specific skin diseases. Knowledge of how various layers of skin adhere to one another is now being applied to the development of more effective means for treating chronic wounds, but heretofore the effect of oxygen supply at the wound site has been unclear.

Advance: Low oxygen tension (the amount of oxygen available) has been shown to stimulate cell growth and the synthesis of certain growth factors and components important in wound healing. In a recent study, investigators used a tissue culture model to simulate acute wounding. They analyzed oxygen tension and found that it dropped in the areas of model wounds as compared to the unwounded areas, but the decrease was blocked by inhibitors of cellular protein synthesis. The researchers concluded that the drop in oxygen tension is the result of increased cellular activity as a wound-healing response rather than as the cause of the wound.

Implications: These findings suggest that improving circulation to deliver oxygen to wounds would be beneficial to healing, as would the use of hyperbaric (high pressure) oxygen and other treatments designed to increase oxygen supplied to the tissue from outside.

Tokuda Y, Crane S, Yamaguchi Y, Zhou L, Falanga V: The levels and kinetics of oxygen tension detectable at the surface of human dermal fibroblast cultures. Journal of Cellular Physiology, 182(3):414-20. 2000.

Delaying Surgery to Replace Osteoarthritic Knees Results in Worse Functional Status

Background: Total hip replacement and total knee replacement have revolutionized the treatment of disabling lower extremity osteoarthritis (OA). Approximately 270,000 of these procedures are performed annually in the United States. Although 90 percent of patients experience substantial pain relief, total joint replacement is not without risks. The ideal point at which to perform surgery in the course of osteoarthritis has not been defined. Traditional orthopaedic practice has been to delay surgery until pain and functional limitation are intolerable. It has been suggested, however, that earlier surgery may decrease the length of stay in hospital and prevent loss in quality of life and function.

Advance: Researchers contacted candidates for elective total knee or total hip replacement surgery at two sites: one in Boston and one in Montreal. Canadian patients had worse preoperative status, reflecting limited access to elective total hip or knee replacement for OA. All patients who entered the study completed a questionnaire preoperatively and at 3 and 6 months postoperatively. Results suggest that the single best predictor of pain and function at 6 months after total hip or knee replacement is the subject's baseline pain or function. Advanced functional loss due to OA of the hip or knee was associated with worse outcome at 6 months. Patients with worse preoperative function remained significantly worse postoperatively. Independent of baseline pain or function, a higher education level was associated with less pain and better function at 6 months after surgery.

Implications: Patients with worse preoperative quality of life and physical function and more pain continued to have more pain and worse function postoperatively than their counterparts with better preoperative status. These differences were striking, clinically relevant, and more marked for total knee replacement. The cost and modest risk associated with total joint replacement appear to be offset by an improved quality of life when the surgery is performed prior to advanced functional loss.

Fortin PR, Clarke AE, Joseph L, et al: Outcomes of total hip and knee replacement: preoperative functional status predicts outcomes at 6 months after surgery. Arthritis & Rheumatism, 42(8):1722-8. 1999.

Liposome-Mediated Gene Transfer Can Be Used to Treat Basal Cell Skin Cancer

Background: Skin is an ideal organ for gene therapy because of its accessibility. Particularly noteworthy is the opportunity it affords for introducing genetic material using vehicles other than viruses (there is concern that viruses could somehow become active again and be infectious). The two major mechanisms of introducing genetic material are by direct injection or by the use of carrier molecules such as liposomes (synthetic fat complexes).

Advance: Basal cell skin cancer is the most common cancer in the United States. In an animal model in which the human cancer was transplanted to an immune-deficient mouse, researchers injected DNA complexed to liposomes. The DNA induced the production of interferon, and the interferon induced the reduction of the skin cancer.

Implications: This finding has potential for direct human application in the nonsurgical treatment of basal cell skin cancer. Furthermore, it demonstrates that gene therapy is feasible in the skin in the absence of viral vectors. Possibly, a gene cream could be developed.

Hottiger MO, Dam TN, Nickoloff BJ, Nabel GJ: Liposome-mediated gene transfer into human basal cell carcinoma. Genetic Therapy, 6(12):1929-35. 1999.

Bone-to-Bone Attachment May Be the Best Method for Reconstructive Hip Surgery

Background: Loss of bone in the femur is a significant problem after injury, infection, tumor, or osteolysis (bone degradation) following total hip replacement. Several surgical techniques are available, but inadequate or failed attachment of the muscles that connect the hip to the grafted tissue/prosthesis is a common problem.

Advance: To examine the long-term, time-related changes that occur during incorporation of tendon and bone following femoral reconstruction, researchers performed surgeries on 24 adult, mixed-breed dogs. They used three surgical reconstructive methods: tendon to tendon, tendon to bone, and bone to bone. X rays revealed that bone-to-bone attachment had the best functional outcome after 9 months.

Implications: Although direct extrapolation of canine findings to humans should be made with care, the forces transmitted across the hip are similar between humans and dogs, and the dog is a known clinically applicable model for total hip reconstruction. The results of this study indicate that the bone-to-bone method should be used for tendon attachment whenever possible during hip revision surgery.

Pluhar GE, Heiner JP, Manley PA, Bogdanske JJ, Vanderby R, Manley MD: Comparison of three methods of gluteal muscle attachment to an allograft/endoprosthetic composite in a canine model. The Journal of Orthopedic Research, 18(1):56-63. 1999.

Improved Treatment for Blood Clots in the Leg

Background: Blood clots (deep vein thrombosis) form in veins of the leg in over 150,000 patients in the United States each year. The current form of treatment, anticoagulation, prevents extension or growth of the blood clot and prevents the clot from breaking off and moving to the lung (pulmonary embolism). Anticoagulation does preserve life by preventing pulmonary embolism but does not preserve the quality of life because over a third of these patients slowly develop (over 5 to 10 years) swelling, chronic skin thickening, and even chronic leg ulcers as a result of damage to the veins in the affected leg. Thus, while anticoagulation appears to give good immediate results, it is an inadequate treatment for restoration of the venous circulation in the leg. Failure of anticoagulation to clear the blood clot rapidly enough to prevent damage to the leg veins and their valves has long been recognized and led a 1980 NIH Consensus Conference to recommend use of thrombolytic enzymes to rapidly dissolve the blood clots in the leg. This recommendation has not been adopted because of safety issues and high costs of the thrombolytic enzymes and of the intensive care hospitalization required for therapy.

Advance: As a result of advances in biotechnology, there is a wider selection of thrombolytic agents or enzymes, but the large volume of blood clot in the leg (over a thousand times larger than the amount of blood clot found in the artery to the heart or brain during heart attack or stroke) still requires development of more efficient use of these expensive thrombolytic enzymes. Direct (intraclot) injection of the enzyme into the clot was shown to be both more efficient and safer than simple intravenous infusion into the general circulation during the last decade. Even with this more efficient delivery the cost of the thrombolytic enzyme, urokinase, used in these studies was prohibitive ($10,000-$40,000/ leg). By exploiting the clot binding property of another thrombolytic enzyme, recombinant tissue plasminogen activator (r-tPA) researchers believed that thrombolytic treatment could be simplified and made affordable without compromising safety. The enzyme, r-tPA, is forcefully injected throughout the clot using pulse spray catheters only once a day in radiology. Unlike other agents , r-tPA sticks to the clot and does not require continuous replacement, reducing cost and eliminating the need for intensive care hospitalization. As a result, the cost of treatment using r-tPA in this study is more than 10 times lower than reported costs from the previous treatments using urokinase.

Implications: If the cost can be reduced, the combination of thrombolytic therapy and anticoagulation therapy to prevent both pulmonary embolism and impairment of circulation in the leg as originally recommended by the NIH Consensus Conference in 1980 becomes a practical option. Researchers are optimistic that the doses (and costs) of r-tPA can be further reduced and that with the development of low molecular weight heparin that it may be possible to provide treatment without need for hospitalization for most patients. Researchers hope to investigate these possibilities in future investigations.

Horne MK III, Mayo DJ, Cannon RO III, Chen CC, Shawkwer TH, Chang R: Intraclot recombinant tissue plasminogen activator in the treatment of deep venous thrombosis of the lower and upper extremities. The American Journal of

Medicine, 108(3):251-5. 2000.

Widely Used Herbal Remedy, St. John's Wort, Found to Cause Significant Interaction with a Drug for the Treatment of HIV Infection

Background: Herbal remedies are widely used by Americans with an estimated $5 billion dollars spent each year. St. John's wort is a commonly used herbal product often used for the treatment of mild to moderate depression. Since depression is common in HIV-infected patients, St. John's wort is often taken along with drugs for the treatment of AIDS. Herbal remedies are not regulated by the FDA, and so very little information is known about how these products might interact with prescription medications. Study was undertaken to see if St. John's wort could be safely administered along with indinavir, a drug commonly used for the treatment of HIV.

Advance: Researchers at the NIH studied healthy volunteers and discovered that St. John's wort decreased the amount of indinavir in the body by over 50%. This information provided a new understanding that herbal remedies may not always be safe just because they are natural products. This study demonstrated that St. John's wort could cause serious drug interactions in patients who are taking indinavir for HIV infection.

Implications: The U.S. Food and Drug Administration issued a health advisory on St. John's wort drug interactions as a direct result of this study. Five other European regulatory agencies also issued warnings including Great Britain, the Netherlands, and Australia. Companies that make drugs for HIV infection added this information into their product labels. Major manufacturers of St. John's wort also voluntarily agreed to add a statement that patients should seek the advice of health professional before using it with other drugs. This study sparked a great deal of interest in evaluating herb-drug interactions. Several centers in the US and abroad are now beginning research programs in this field. Additionally, this study provided insight into how St. John's wort might affect other prescription drugs. It is thought that St. John's wort speeds up the breakdown of indinavir by the liver. Several drugs are metabolized in the body through the same pathway that indinavir goes through including drugs for epilepsy, heart disease, prevention of transplant rejection, high cholesterol, and oral contraceptives. These medications may also be affected by St. John's wort and health care professionals can now better inform their patients of potential interactions.

Piscitelli SC, Burstein AH, Chaitt D, Alfaro RM, Falloon J: Indinavir concentrations and St. John's wort. The Lancet, 355(9203):547-8. 2000.

The Role of HPA Axis Hyperactivity in Transducing Childhood Abuse into Adult Psychopathology

Background: Over the past two decades, data have been accrued linking early adverse life experiences (for example, abuse, parental loss) to the subsequent emergence in adults of mood and anxiety disorders, both of which are substantially more prevalent in women. During this same timeframe, neuroscience investigations have increasingly implicated corticotropin releasing factor (CRF) systems as a prime biological mediator of this relationship. CRF, a peptide found in several regions of the brain, including the hypothalamus, amygdala and neocortex, activates the pituitary-adrenal "stress axis." Although several animal studies strongly suggest increased activity of CRF circuits as a persisting neurobiological consequence of early life stress, this hypothesis had not previously been tested in a human population.

Advance: NIH-supported researchers studied 4 groups of women, ages 18 to 45 years. The groups were: (1) Normal controls; (2) women with a diagnosis of current major depression and a history of childhood physical or sexual abuse; (3) women without current major depression but with a history of childhood physical or sexual abuse; and (4) women with a diagnosis of current major depression without any history of childhood physical or sexual abuse. All subjects participated in a standard psychosocial stress protocol (essentially a 10-minute anticipation and preparation phase followed by a 10-minute public speaking and mental arithmetic task in front of an audience). During this "stress exercise," the investigators obtained blood samples to measure pituitary and adrenal "stress hormones" (ACTH and cortisol) and heart rate measurements. Women with a history of childhood abuse exhibited increased responses to stress compared with controls. This effect was particularly robust in women with current symptoms of depression and anxiety. Women with a history of childhood abuse and a diagnosis of current major depression exhibited a more than 6-fold greater ACTH response to stress than age-matched controls. There were also significant differences in cortisol levels and response as well as heart rate responses.

Implications: This is the first clinical study to report persistent changes in stress reactivity in adult survivors of early trauma. Taken in conjunction with very similar results in studies with laboratory animals, these findings suggest that exaggerated reactivity of the hypothalamic-pituitary-adrenal axis and autonomic nervous system, presumably due to CRF hypersecretion, is a persistent consequence of childhood abuse that may contribute to a predisposition for adult psychopathology. These results may imply a role for medications that would block CRF receptors for use in treating and preventing mental disorders related to early-life stress.

Heim C, Newport DJ, Heit S, Graham YP,et al: Pituitary-adrenal and autonomic responses to stress in women after sexual and physical abuse in childhood. Journal of the American Medical Association, 284(5):592-7. 2000.

New Insights into Lithium's Beneficial Effects in Bipolar Disorder

Background: Lithium is the main pharmacological treatment for bipolar disorder. When taken regularly, lithium can effectively stabilize mania and depression in many patients and can reduce the likelihood that episodes of illness will recur. However, after more than three decades of use in the U.S., scientists still do not understand exactly how lithium exerts its beneficial effects, nor do they understand why it works well for some people, but not others. In an attempt to answer these questions, researchers have investigated the biochemical mechanisms of action of lithium.

Advance: Several NIH-supported projects have shown that lithium can positively affect the way nerve cells survive, change their shapes and metabolic rates, and form connections with other nerve cells. This unexpected action of lithium in maintaining and protecting the health of cells may have a significant impact on our understanding of its therapeutic mechanism of action. In one study, after 4 weeks of chronic lithium administration, researchers measured a robust increase in the levels of an important neuroprotective protein, called bcl-2, in several regions of the rat brain (cortex, hippocampus, and striatum). Similar lithium-induced increases in bcl-2 were seen in human neuronal cells in culture. These are highly significant findings because bcl-2 not only protects neurons against cell death, whether death is programmed or induced by diverse insults, but it also promotes the regeneration of axons in the central nervous system (CNS).

The effects of lithium on bcl-2 are consistent with earlier observations of lithium's ability to inhibit the activity of an enzyme that regulates cell metabolism and contributes to programmed cell death. The next step was to determine if the increases in bcl-2 levels and the lithium's inhibition of this enzyme had functional consequences. Two teams of researchers demonstrated that lithium did protect neurons from the deleterious effects of experimental insults, such as glutamate toxicity in rats and heat shock in neuronal cell culture.

Researchers have now shown that lithium exerts protective and positive effects in human brain. Using non-invasive proton magnetic resonance spectroscopic (MRS) imaging, researchers assessed the viability and function of neurons in several cortical regions in patients with bipolar affective disorder before and after 4 weeks treatment with lithium. Chronic lithium treatment was shown to increase a brain chemical that is indicative of cellular health in both the frontal and temporal cortex, in both patients with bipolar disorder and normal control subjects.

Implications: This robust evidence of lithium's neuroprotective properties at therapeutic concentrations provides the groundwork for larger, longitudinal studies to determine if lithium reduces or delays neuronal cell death and atrophy in patients with bipolar disorder. Documentation of lithium's neuroprotective effects, coupled with findings that mood disorders can be associated with cell loss and atrophy, suggests beneficial effects of lithium in the long-term treatment of mood disorders. This work will lead to the identification of new cellular and molecular targets of lithium's actions and will inform the

development of new and better treatments for bipolar disorder.

Manji HK, Moore GJ, Chen G: Lithium up-regulates the cytoprotective protein bcl-2 in the CNS in vivo: a role for neurotrophic and neuroprotective effects in manic depressive illness. Journal of Clinical Psychiatry, 61(supplement 9): 82-96. 2000.

Bijur GN, De Sarno P, Jope RS: Glycogen synthase kinase-3 beta facilitates staurosporine- and heat shock-induced apoptosis. Protection by lithium. The Journal of Biological Chemistry, 275(11):7583-90. 2000.

Moore GJ, Bebchuk JM, Hasanat K, Chen G, Seraji-Bozorgzad N, Wilds IB, Faulk MW, Koch S, Glitz DA, Jolkovsky L, Manji HK: Lithium increases N-acetyl-aspartate in the human brain: In vivo evidence in support of bcl-2's neurotrophic effects? Biological Psychiatry, 48(1):1-8. 2000.

Anti-anxiety Pills Don't Work For You? Maybe This Is Why

Background: Low serotonin receptor levels are found in severe stress, and in many mood and anxiety-related disorders (for example, panic attacks). Medications used to treat these disorders typically increase levels of neurotransmitters, such as serotonin. A particular class of medications, called benzodiazepines (BZ), of which Valium is a prototype, is usually very effective at reducing anxiety. These drugs act at a receptor called the $GABA_A$ receptor and improve its functioning. A small percentage of people do not get relief from BZ medications, however, and the reason has been unclear.

Advance: Scientists developed a model of anxiety by "knocking out," or deleting, a particular serotonin receptor in mice. These "model" mice with the missing serotonin receptors displayed symptoms of anxious behavior, but did not respond to the "relaxing" effects of a BZ compound. This behavior thus modeled that of humans with anxiety disorders who did not respond to BZ. For example, the "knockout" mice spent less time in the open and more time in a "protective" environment than is usual when BZ is administered. They also required three times more BZ than normal mice in order to reduce their locomotor activity and were less sensitive to the anesthetic effects of drugs.

The site of action of BZ is a brain region called the amygdala that is important in fear and emotional responses. This study found that the amygdala in the serotonin receptor knockout mice, as compared with normal mice, contained fewer $GABA_A$ receptor subunits that respond to BZ. The research also demonstrated that levels of certain $GABA_A$ subunits in the amygdala and other brain regions are under the control of the serotonin receptor system. The finding that inactivation of the serotonin receptor causes changes in the $GABA_A$ receptor, making it less likely to respond to BZ, suggests that patients with fewer or non-functioning serotonin receptors likely will not respond to BZ treatment.

Implications: The underlying basis for a given disorder may vary from person to person. Common overt behaviors associated with anxiety-related disorders can be the result of more than one complication (for example, a low transmitter level or a non-functional receptor, etc.). Treatment would differ depending on where the problem is located. This study identifies specifically which components of a brain transmitter system are likely to be involved in anxiety. The information affords basic scientists a specific therapeutic target for developing drugs to help BZ-insensitive individuals and, more immediately, provides clinicians a compelling rationale for prescribing currently available drug alternatives to BZ-insensitive individuals.

Sibille E, Pavlides C, Benke D, Toth M: Genetic inactivation of the serotonin receptor in mice results in downregulation of major $GABA_A$ receptor alpha subunits, reduction of $GABA_A$ receptor binding, and benzodiazepine-resistant anxiety. The Journal of Neuroscience, 20(8):2758-65. 2000.

Translational Research Suggests Enhanced Treatment Strategy in Schizophrenia

Background: Among the most prominent clinical features of schizophrenia are cognitive deficits and negative symptoms, which are marked by social withdrawal and dulled emotional responsiveness. In some cases, these symptoms may be worsened by the very medications used to treat the underlying disorder. The efficacy of standard antipsychotic drugs correlates to their ability to block a particular receptor for the neurotransmitter dopamine, the D2 receptor. These medications also induce *down-regulation* – that is, restrict the availability – of another dopamine receptor subtype, the D1 receptor, that is essential to working memory, a very short-term form of recollection for immediate past events. Recently, translational investigators supported under a NIMH Conte Neuroscience Research Center grant reported a preclinical study that explored a possible mechanism for these effects and suggests novel strategies for clinical treatments.

Advance: The researchers examined the effects of antipsychotic drugs on working memory in young, adult, non-human primates. Over a period of 6 to 12 months, monkeys were trained on tasks of spatial and object working memory. Then, over an extended period, the monkeys were administered "clinically effective" doses (that is, 5-15 mg/day) of the antipsychotic drug, haloperidol, before being retested on the same memory tasks that they had previously mastered. After receiving the medication for 1 to 4 months, significant impairment on both working memory tasks was evident. The monkeys' performance on two control tasks that involved object retrieval and fine motor performance indicated that the working memory deficits were independent of the sensory or motor components of these tasks. Because cognitive deficits emerged within the timeframe of expected D1 receptor *down-regulation*, the investigators hypothesized that the working memory deficits observed resulted from such *down-regulation*. To test this hypothesis, a D1 agonist – a compound that selectively boosted functional availability of D1 receptors – was administered for a series of five to six 5-day periods, each period alternating with a 2-week period without the agonist. During 3-7 months of this intermittent co-administration of D1 agonist, all monkeys displayed a significant improvement in their performance on the spatial working memory task (and a non-significant, but strong, trend of improvement on the object working memory task). With repeated exposure to the agonist, the monkeys showed increasingly extended periods of sustained improvement on the working memory tasks, which carried over into the washout periods. After the final treatment, performance did not differ significantly from the pre-haloperidol baseline in most cases. This reversal persisted, in some cases, for more than 1 year.

Implications: The finding that chronic haloperidol treatment can induce cognitive impairments that can be reversed by short-term D1 stimulation has potential relevance for the treatment of cognitive deficits and/or negative symptoms in a variety of conditions. D1 *down-regulation* in the prefrontal cortex has been reported in both unmedicated and medicated schizophrenic patients. The recovery produced by brief periods of co-administration of D1 agonist demonstrated in this study suggests that patients may show improvements in their cognitive abilities with concurrent use of antipsychotic medications and an auxiliary D1 agonist.

Castner SA, Williams GV, Goldman-Rakic PS: Reversal of antipsychotic-induced working memory deficits by short-term dopamine D1 receptor stimulation. Science, 287(5460):2020-2. 2000.

"Chili Pepper Receptor" A Target For Pain-Reducing Medications

Background: Sensations that we experience as "painful," whether they result from physical forces, such as heat or pressure, or chemical causes, such as acids, are detected by specialized neurons that carry information to the spinal cord and brain. Sensations so extreme as to cause tissue damage activate special receptor molecules on these neurons. Imagine the pain that occurs when you bite into a red hot chili pepper. The ingredient that makes the chili pepper hot is called capsaicin. It binds to specific receptors, named VR1 receptors, that are located on nerve endings of pain-sensing cells. Because this "hot pepper receptor" appears to be critical in detecting noxious stimuli, scientists reason that identifying precisely which types of stimuli VR1 receptors detect will help them tailor effective clinical treatments to specific pain-related injuries.

Advance: Working with mice, NIH-supported investigators disabled the gene that expresses, or produces, the VR1 receptor molecule and, thus, eliminated the animals' sensitivity to capsaicin and related compounds. Mice lacking the VR1 receptor could freely drink water containing capsaicin, but mice with the receptor intact would take one taste, rub their snouts, and drink no more. When it comes in contact with skin, capsaicin normally elicits behavioral responses such as paw-licking and paw-shaking. Here again, however, mice lacking the VR1 receptor showed little or no behavioral responses. Moreover, consistent with the idea that VR1 helps to activate additional inflammatory agents, the "swelling response" of the VR1-deprived mice was significantly reduced as compared with their VR1-intact counterparts.

These studies also suggested that the role of VR1 in conveying pain messages is stimulus-specific. Mutant animals tolerated heat increases better than control mice, and, unlike controls, did not demonstrate hypersensitivity in inflammation caused by application of mustard oil. However, both mutant and normal mice demonstrated increased sensitivity to being touched or rubbed following the mustard oil treatment, indicating that the VR1 receptor does not have a role in touch-or pressure-related pain.

Implications: Injured and inflamed tissues are at risk for exciting and activating pain receptive neurons. The finding that sensory neurons in VR1-deficient mice show dramatically reduced excitation in a typical inflammatory acidic environment suggests that VR1 might be a potential target for medications designed to alleviate chronic pain in conditions of tissue injury and related inflammation. Given the discrete localization of VR1 receptors and their highly specialized responses, new pharmaceutical products that target the receptor may have few of the side-effects often found in anti-pain medications.

Caterina MJ, Leffler A, Malmberg AB, et al: Impaired nociception and pain sensation in mice lacking the capsaicin receptor. Science, 288(5464):306-13. 2000.

Treatment of Negative Symptoms in Schizophrenia

Background: Schizophrenia is the most serious of mental disorders, striking typically in young adulthood and devastating the lives of patients and family members. While the "positive" symptoms of schizophrenia (hallucinations and delusions) are the most dramatic manifestation of illness, the so called "negative" symptoms (social withdrawal and apathy) are often the most disabling. Currently available antipsychotic treatments, which work mostly through blocking the neurotransmitter, dopamine, are generally quite good in managing "positive" symptoms, but have limited effects on "negative" symptomatology. In recent years, scientists have hypothesized that decreased activity of another neurotransmitter, glutamate, at the NMDA receptor in the brain, is associated with negative symptoms in schizophrenia.

Advance: In two separate studies supported by NIH, the investigators studied the effects of agents, called agonists, that increase the activity of the NMDA receptor in patients with schizophrenia. An agonist is a compound that stimulates physiologic activity at receptors that normally are stimulated by naturally occurring brain chemicals. Because the NMDA receptor requires glycine to function normally, one group of scientists used D-cycloserine, which is a partial agonist at the glycine-modulatory site of the NMDA receptor. Another group used high doses of glycine, itself. In double-blind, placebo-controlled, randomized, clinical trials, both D-cycloserine and glycine improved negative symptoms in patients with schizophrenia significantly more than placebo.

Implications: While the number of subjects was small, and the results must be taken as preliminary, these two studies strongly suggest: (1) It is possible to target negative symptoms in schizophrenia, and (2) NMDA receptor function may be related to negative symptomatology in schizophrenia. For a variety of reasons, D-cycloserine and glycine may not be viable long-term treatments, themselves. Nevertheless, this "proof-of-concept" serves as a first step and a prod to further research to develop treatments for negative symptoms in schizophrenia.

Goff DC, Tsai G, Levitt J, et al: A placebo controlled trial of D-cycloserine added to conventional neuroleptics in patients with schizophrenia. Archives of General Psychiatry, 56(1):21-7. 1999.

Heresco-Levy U, Javitt DC, Ermilov M, et al: Efficacy of high-dose glycine in the treatment of enduring negative symptoms of schizophrenia. Archives of General Psychiatry, 56:29-36. 1999.

Genomic Integration Via a Hybrid Adeno-Retroviral Vector

Background: The overall goal of gene therapy is to treat diseases by replacing or repairing non-functional DNA. To deliver therapeutic genes to target tissues requires a vector, or vehicle, to carry them. Desirable characteristics of a gene therapy vector include highly efficient entry into target cells and long-term gene expression, or function. None of the available vector systems meet these requirements. The retrovirus called Moloney murine leukemia virus (MoMLV) was one of the first viral vectors used for human gene therapy. The advantages of MoMLV are its efficient entry into dividing cells, and the integration of transferred genetic material into target cells, which provides the opportunity for stable gene function. However, MoMLV does not efficiently enter or integrate into non-dividing cells and it is not possible to produce the retrovirus in large quantities. Conversely, adenoviruses, or common cold viruses, can successfully enter many cell types with high efficiency and can also be produced in large quantities. But adenoviruses do not integrate into the target cell genome and this can result in unstable gene function. By combining adenoviral and retroviral elements, scientists theorized they could create a hybrid vector that would offer superior performance.

Advance: NIH researchers created a new hybrid vector by taking two pieces of the retrovirus MoMLV and inserting them into an adenovirus. In cell culture and animal studies, the new hybrid was successfully integrated into the genome of a variety of cell types, both dividing and non-dividing. Additionally, the animal studies showed that the transferred genetic material could function for as long as three months.

Implications: This highly efficient vector that can be produced in large quantities may have broad applications for human gene therapy.

ZhengC, Baum BJ, IadarolaMJ, O'Connell BC: Genomic integration and gene expression by a modified adenoviral vector. Nature Biotechnology, 18(2):176-80. 2000.

Scientists Find Potential Way to Improve Gene Therapy Delivery to Lungs and Brain

Background: The efficient delivery of therapeutic genes to targeted cells is a key step in the development of gene therapy. Recombinant AAV2 (adeno-associated virus) has been widely studied as a vector for transferring genes to a variety of cells *in vitro*, and to several organs *in vivo*. But AAV2 has its limitations – it is only able to get inside of (transduce) certain cell types. In fact, AAV2 is inefficient in transducing the apical surface of human airway epithelia, which play a critical role in the genetic disease cystic fibrosis. AAV2 may also be of limited value in delivering treatment for neurodegenerative disorders such as Alzheimer's disease, since animal studies show that AAV2 is only effective locally at the site of injection in the brain. To treat neurodegenerative disorders, a vector must be able to spread out and deliver correct copies of genes to cells throughout the central nervous system. AAV2 is classified as a dependent parvovirus of which a total of 6 primate isolates have been reported, referred to as AAV1-6. Differences in the protein coats suggest that some isolates may be able to transduce different cell types. Initial *in vitro* experiments by NIH scientists have supported this idea for AAV4 and AAV5.

Advance: As a result of these differences, NIH scientists, in collaboration with the University of Iowa, tested AAV5 as a vector for gene therapy in the lung, and AAV4 and AAV5 as vectors for delivering genes to the central nervous system. Their studies show that AAV5 is significantly better than AAV2 in transducing several cell types in the lung, both in laboratory studies of human lung cells and in animal studies using mice. Working with mice, the scientists also studied the ability of AAV4 and AAV5 to transfer genes in the brain. In this study, AAV4 turned out to be very specific and able to transduce just one cell type in the brain – the ependyma, which gives rise to other cell types and also forms a layer surrounding the ventricle in the brain. AAV5 on the other hand, reached not only the ependyma, but also other cell types including neurons. Furthermore, the AAV5 vector had a unique ability to spread far beyond the injection site after being introduced into the brain.

Implications: Studies in mice confirm the initial laboratory observations that AAV4 and AAV5 are more efficient vectors than AAV2 for gene transfer because of their improved ability to get into targeted cells. AAV5 vectors containing therapeutic genes may be useful in treating a variety of diseases such as cystic fibrosis and _lpha-antitrypsin deficiency in the lung. AAV4 and AAV5 vectors may prove useful for treating neurodegenerative disorders such as Parkinson's disease, Alzheimer's disease, and Huntington's disease.

Davidson BL, Stein CS, Heth JA, et al: Recombinant adeno-associated virus type 2, 4, and 5 vectors: transduction of variant cell types and regions in the mammalian central nervous system. Proceedings of the National Academy of Sciences, 97(7):3428-32. 2000.

Zaber J, Seiler M, Walters R, et al: Adeno-associated virus type 5 (AAV5) but not AAV2 binds to the apical surfaces of airway epithelia and facilitates gene transfer. Journal of Virology, 74(8):3852-8. 2000.

Possible New Therapy for Leber's Congenital Amaurosis

Background: In 1869 Theodor Leber described an early-onset recessive retinal degeneration that caused incurable blindness in children. This disease became known as Leber's congenital amaurosis (LCA). For many childhood genetic diseases like LCA no treatment is currently available. The necessary gene is either missing or defective. With recent advances in our understanding of the basis for genetic diseases, scientists have been able to identify defective genes that are associated with specific diseases. Once the gene is identified, however, the patient is still faced with the prospects of no immediate cure. Such was the case for LCA, when in 1997 the disease-causing mutations in a gene known as RPE-65 were linked to an estimated 10% of LCA cases. Fortunately, recent animal research offers the hope that vision may one day be restored to some patients with LCA.

Advance: One way to understand a disease is to duplicate it in an animal model. Recently, NIH scientists have produced mice lacking the RPE-65 gene. This lack of the RPE-65 gene produces a defect in the visual cycle, a series of biochemical events in the light-sensing retina that initiate vision. The defect eventually results in impairment of photoreceptor cell function and retinal degeneration. It was known that the protein product of the RPE-65 gene participates in the visual cycle. In order to better understand the function of RPE-65, scientists studied the individual components of the visual cycle pathway and found that RPE-65 is involved in a biochemical reaction called an isomerization. Thus, the mice lacking RPE-65 allowed scientists to focus on the possible function of this molecule. Next, a way was found to bypass the defect in the visual cycle. For this, RPE-65 deficient mice were fed a form of vitamin A called 9-cis-retinal. This chemical is not normally found in photoreceptor cells but it forms part of the functional visual pigment isorhodopsin. The result was dramatic and resulted in improved photoreceptor physiology and function.

Implications: Administration of compounds like 9-cis-retinal for LCA is an example of pharmacological interventions that could restore vision and relieve the suffering and burden caused by some blinding diseases. Animal studies with 9-cis-retinal have opened the door to studies in humans that may contribute to the development of improved treatments.

Van Hooser JP, Aleman TS, He Y, et al: Rapid restoration of visual pigment and function with oral retinoid in a mouse model of childhood blindness. Proceedings of the National Academy of Sciences, 97(15):8623-8. 2000.

Cytomegalovirus Retinitis in AIDS Patients Treated with Highly Active Antiretroviral Therapy (HAART)

Background: Cytomegalovirus (CMV) retinitis is a potentially blinding AIDS-related eye complication. Standard therapy to preserve vision has heretofore been life-long use of a variety of anti-CMV drugs. In addition to being expensive, these drugs can have serious side effects, including kidney toxicity and low blood cell counts, and may require daily intravenous administration.

Advance: A new treatment protocol has been developed that appears to help rejuvenate the immune systems of human immunodeficiency virus (HIV)-infected individuals. This treatment is known as highly active antiretroviral therapy or HAART and employs a combination of anti-HIV drugs. NIH-supported researchers have found that patients receiving HAART were able to stop taking their standard anti-CMV medications safely without further progression of their retinitis. In addition to the visual benefits derived from keeping the CMV retinitis from progressing, the study suggests that HAART causes a sustained recovery of the immune system such that it may be able to control other opportunistic infections in people with AIDS.

Implications: The use of the orally administered HAART will change clinical practice and lead to a significant improvement in the quality of life for people with AIDS who have CMV retinitis.

Whitcup SM, Fortin E, Lindblad, AS, et al: Discontinuation of anticytomegalovirus therapy in patients with HIV infection and cytomegalovirus retinitis. The Journal of the American Medical Association, 282(17):1633-7. 2000.

Macdonald JC, Karavellas MP, Torriani FJ, et al: Highly active antiretroviral therapy-related immune recovery in AIDS patients with cytomegalovirus retinitis. Ophthalmology, 107(5):877-883. 2000.

Identification of a New Class of Compounds that Slows Development of a Prion Disease in Mice

Background: Transmissible spongiform encephalopathies (TSE) are a group of degenerative, invariably fatal brain disorders in animals and humans. The most common TSE diseases are scrapie in sheep, bovine spongiform encephalopathy or "mad cow disease" in cattle, and Creutzfeldt-Jakob disease in humans. The infectious agent that causes TSE diseases, called a prion (for "proteinaceous infectious agent") is associated with an aberrant form of a normal cellular protein, PrP. Normal PrP is folded into a distinct three-dimensional structure and is designated as PrP-sen because it is sensitive to destruction by proteases, enzymes that cut proteins into fragments. Abnormal disease associated PrP, which is associated with infectious prions, is designated PrP-res because it is resistant to destruction by proteases. PrP-res may arise spontaneously by mutation or may be transmitted from animal to animal, or animal to human, through a mechanism that is poorly understood. Within an infected individual, PrP-res forces PrP-sen to assume the shape of PrP-res, although the biological mechanism behind this pathological transformation has not yet been identified. When an animal or person develops TSE, this process leads to a build up of abnormal prion protein (i.e., PrP-res) in the brain, disrupting neuron function. As cells die off, brain tissues become filled with holes, resulting in the "spongy" appearance characteristic of these diseases. Symptoms include progressive dementia and a loss of muscle control, followed by coma and death. At present, there is no cure for any form of TSE.

Advance: Researchers have discovered that a family of chemical compounds known as cyclic tetrapyrroles can significantly slow disease progression in mice experimentally infected with scrapie. Large doses of scrapie were injected into mice genetically engineered to overexpress hamster PrP-sen. When given at the time of scrapie injection, or mixed with the injection, cyclic tetrapyrroles increased survival time dramatically, in some cases by 300 percent. However, when cyclic tetrapyrroles were given to mice at a later stage of disease progression, they had only a minimal effect. The cyclic tetrapyrroles appear to interfere with the conversion of PrP-sen to PrP-res by binding with PrP-sen, PrP-res, and/or an intermediate PrP-sen/PrP-res complex. By significantly impairing the transformation of Prp-sen into PrP-res the cyclic tetrapyrroles delay the onset of disease.

Implications: These results suggest that cyclic tetrapyrroles might delay disease if administered prior to, or early during, natural infection. In addition, they might provide an effective means for de-activating infectious prion proteins in contaminated biological materials. Although the specific cyclic tetrapyrroles tested are unlikely to be effective in treating people who already have clinical disease symptoms, researchers are optimistic that other cyclic tetrapyrroles could slow disease progression in these patients. The discovery also advances current knowledge of TSE pathogenesis. Determining the mechanisms through which normal proteins are transformed into pathological proteins should lead to a better understanding of more common diseases of abnormal protein folding, including Alzheimer's disease and type 2 diabetes, and offer new strategies for combating these disorders.

Priola SA, Raines A Caughey WS: Porphyrin and phthalocyanine antiscrapie compounds. Science, 287(5457):1503-6. 2000.

Therapies for Treating Infants with Severe Combined Immunodeficiency Show Long-lasting Benefits

Background: In a landmark study by French investigators, gene therapy successfully corrected the defect in two infants with severe combined immunodeficiency disease (SCID). SCID, commonly known as the 'bubble boy syndrome," is characterized by a lack of functioning T cells and B cells resulting in the immune system's inability to prevent life threatening infections. Although multiple gene defects can result in SCID, most often SCID is due to loss of a functioning gamma chain, a component common to multiple cytokine receptors. Three months after re-infusion of their own stem cells, into which a normal gamma chain gene had been inserted, two patients who received this type of gene therapy no longer required protective isolation. The only other therapy for SCID is bone marrow transplantation, provided a suitably matched donor can be found. In this procedure, bone marrow stem cells are transfused, and subsequently develop into mature T cells and B cells. A central question concerning the success of both gene therapy and bone marrow transplantation is the durability of the immune response. In both cases, T cell precursors require residency in the thymus gland to develop into mature functional cells. Since the thymus in SCID patients is underdeveloped, its ability to sustain T cell development over a lifetime is unknown.

Advance: In separate work, a team of NIH-supported investigators studied 83 SCID patients who had undergone bone marrow transplantation to receive stem cells over an 18-year period. T cells that developed in each patient's thymus were identified, characterized, and followed over time with molecular and cellular markers. The number of T cells reconstituted in the thymus peaked to near normal levels at 1-2 years after transplantation, with continued thymic function for up to 14 years.

Implications: Although the decline in thymic function over time was steeper than in normal individuals, the vestigial thymus of SCID patients has the capacity to support T-cell reconstitution for an extended period. These findings suggest that the promising responses to gene therapy, which has the potential to correct the defect in all patients, and donor stem cell transplantation for SCID will be long lived.

Patel DD, Gooding ME, Parrott RE, et al.: Thymic function after hematopoietic stem-cell transplantation for the treatment of severe combined immunodeficiency. The New England Journal of Medicine, 342(18):1325-32. 2000.

How Do Cancer Cells Resist Radiation Treatment?

Background: In cancer, particularly in melanomas, the resistance to radiation is a major handicap for successful treatment. The mechanisms and genes involved in resistance to radiation are not well identified. Russian and U.S. researchers previously discovered that a transcription factor, ATF2, that binds to the UV response element plays a major role in apoptosis induced by the radiation in normal cells. The resistance to radiation develops when the cells do not undergo apoptosis upon exposure to UV or ionizing radiation. The investigators have found that another enzyme, PI3K, which is involved in phospholipid metabolism, may also play a role in transducing the signal from membrane to the nucleus. In these studies the investigators explored the role of PI3K in resistance of cancer cells to radiation.

Advance: The investigators found that the transcription factors PI3K and ATF2 complement each other's contribution to melanoma resistance to radiation. In addition they identified another major signaling component, STAT, which acts as a suppressor of Fas expression, and that such suppression coincides with the ability of late stage melanoma cells to resist radiation.

Implications: The findings of the complementarity in UV resistance between the transcription factors PI3K, ATF and another signaling component (STAT) are major advances in understanding the resistance of some cancer cells to UV radiation treatment. This understanding has direct therapeutic value.

Krasilnikov M Adler V, Fuchs SY, et al: Contribution of phosphatidylinositol 3-kinase to radiation resistance in human melanoma cells. Molecular Carcinogenesis , 24(1):64-9. 1999.

Ivanov VN, Kehrl JH, Ronai Z: Role of TRAF2/GCK in melanoma sensitivity to UV-induced apoptosis. Oncogene, 19(7):933-42. 2000.

Viral Load Associated with Increased Risk of HIV Transmission

Background: Understanding what factors influence the heterosexual transmission of HIV infection is key to developing interventions to prevent transmission. While the amount of HIV virus in a person's body has long been suspected to influence transmission, the contribution of this influence in relation to other factors was not well documented.

Advance: Scientists from the United States and Uganda prospectively studied 415 Ugandan couples in which one partner was HIV positive and the other was not infected with the virus. These researchers found a directly proportional relationship between the amount of virus in an HIV positive individual's blood – or "HIV viral load"– and the likelihood that their sexual partners will contract the virus. They concluded that viral load was a primary predictor of transmission and that infected individuals with levels below 1500 copies of HIV-1 RNA per milliliter rarely passed the disease to their sexual partners.

Implication: These findings document for the first time that viral load directly affects HIV-1 transmission. Therefore, drugs or other methods that reduce the viral load in an infected person will be useful in preventing infection among their uninfected sexual partners.

Quinn TC, Wawer MJ, Sewankambo N,et al: Viral load and heterosexual transmission of human immunodeficiency virus type 1. Rakai project study group. The New England Journal of Medicine, 342(13):921-9. 2000.

HIV and Tuberculosis: Novel Approaches to Treatment

Background: While co-infection with HIV and *M. Tuberculosis* is common in developing countries, the effect of one infection on the other and on progression of diseases is not completely understood. Some studies suggest that active tuberculosis (TB) infection alters the progression of AIDS. It is also uncertain whether HIV infected patients are at high risk for a second episode of TB following successful completion of treatment. Two different studies, one in Uganda and another in Haiti attempt to address these questions.

Advance: One study examined the effect of compliance with anti-TB therapy on disease progression and survival in patients with HIV who do not have access to anti retroviral treatment. HIV-infected adults in Uganda, some of whom have TB, were followed for two years. Those with TB were more likely to die within two years of follow-up (28%) than those without tuberculosis (19%). Among adults with more serious HIV infection, as measured by a low CD4 lymphocyte count, the mortality rate was 1.5 times higher among those with TB. This finding was even more striking among those with less serious HIV infections. The mortality risk for this same group increased three times when TB was also present.

In a related study in Haiti, efforts were made to examine whether recurrent TB occurs more commonly among HIV seropositive individuals, and to determine whether post treatment with Isoniazid (INH) decreases this risk. Recurrent TB was more than five times less likely among those HIV positive individuals who received the anti-TB prophylaxis than those who did not (placebo group). Among HIV positive individuals, all recurrent TB occurred in individuals with a history of HIV related symptoms prior to initial TB diagnosis.

Implication: These studies suggest that aggressive identification and treatment of tuberculosis among HIV infected individuals may lengthen lives and may have the greatest impact in the early stages of HIV infection. Furthermore, those with a history of HIV-related symptoms at the time of TB diagnosis may benefit from prophylaxis following a routine course of treatment.

Whalen CC, Nsubuga P, Okwera A, et al: Impact of pulmonary tuberculosis on survival of HIV-infected adults: a prospective epidemiologic study in Uganda. AIDS, 14 (9):1219-28. 2000.

Fitzgerald DW, Desvarieux M, Severe P, Joseph P, Johnson WD Jr, Pape JW: Effect of post-treatment isoniazid on prevention of recurrent tuberculosis in HIV-1-infected individuals: a randomised trial. The Lancet, 356(99240):1470. 2000.

Do "Epidurals" Prolong Labor and Increase Risk of Cesarean Delivery?

Background: Epidural analgesia is accepted as a safe and effective method to relieve pain during childbirth. However, researchers have questioned whether using this method prolongs labor and increases the need for cesarean deliveries or for deliveries that require the use of forceps or other instruments. Previous research to answer this question has been inconclusive. With more than 50 percent of pregnant women using epidural analgesia in the U.S., and its popularity ever increasing, there is a need for well-designed studies to examine the issue.

Advance: NIH researchers, in collaboration with U.S. Army physicians, compared labor duration, cesarean and instrumental delivery rates, and oxytocin administration rate (a drug used to induce labor), in a "natural experiment" that occurred in an Army community hospital. Use of epidural analgesia was very low in this hospital before October 1993. At that time, a change in the military Anesthesia Service's policy resulted in an immediate and dramatic increase (from 1% to 84% in one year) in epidural use. This significant change created an excellent opportunity to assess the differences in delivery outcomes before and after the new policy was adopted. Examining medical records, researchers compared labor times and other factors in women delivering in this hospital, before and after October 1993. Most importantly, the researchers found that, despite the major increase in labor epidural analgesia use, rates of cesarean delivery and for difficult childbirth remained the same. Although the overall use of instruments in delivery did not increase, the use of outlet forceps and vacuum extraction increased after the method became more readily available. Length of the first stage and active phase of labor did not change; however, the second stage of labor was increased by about twenty-five minutes.

Implications: Labor epidural analgesia does not increase the risk of cesarean delivery, nor does it necessarily increase oxytocin use to speed delivery or overall instrument use for difficult childbirth. The length of an active phase of labor appears unchanged; however the second stage of labor is likely prolonged. These results have important clinical implications for obstetric practice and counseling. Millions of pregnant women and their doctors now have the additional knowledge and confidence they need to select the proper anesthesia plan for labor, while easing concerns about increasing the risk for such invasive procedures as cesarean delivery.

Zhang J, Yancey MK, Klebanoff M, Schwarz J, Schweitzer D: Effects of epidural analgesia on the course of labor and delivery: a natural experiment. Obstetrics and Gynecology, 95(4)Supplement 1:S45. 2000.

SCIENCE CAPSULES

Anti-Nausea Medication Shows Promise in Treating a Severe Form of Alcoholism. Ondansetron, a medication already approved by the Food and Drug Administration for treatment of nausea in chemotherapy patients, also appears to be effective in treating early-onset alcoholism; that is, in individuals who became alcoholic before age 25. Early-onset alcoholism, a severe form of the disease, is characterized by abnormalities in activities involving serotonin, a chemical in the brain that triggers critical functions in nerve cells, the sites of alcohol's actions. Ondansetron blocks a specific kind of nerve-cell receptor for serotonin (the 5-HT_3 receptor). In a randomized trial, the medication significantly reduced alcohol consumption and increased abstinent days among patients with early-onset, but not late-onset, alcoholism. Pending further studies, Ondansetron might be indicated for early-onset alcoholism patients resistant to behavioral therapies alone.

Johnson BA, Roache JD, Javors MA, et al: Ondansetron for reduction of drinking among biologically predisposed alcoholic patients: a randomized clinical trial. The Journal of the American Medical Association, 284(8):963-71. 2000.

Treating Cocaine Addiction with an Ancient Chinese Therapy. Cocaine addiction is a significant public health problem associated with serious medical, psychiatric, social, and economic consequences. There are no medications currently approved for treatment of cocaine addiction. Researchers are investigating the possibility of using acupuncture as a treatment for cocaine addiction. Eighty-two patients addicted to cocaine participated in the study. They were assigned to one of three groups: auricular acupuncture; a control condition where a needle was inserted into the ear where it likely wouldn't generate an effect; or a no-needle relaxation control where the patients viewed relaxation videos. Among the patients who completed the study, more than half of the acupuncture patients tested free of cocaine the last week of treatment compared to 23.5% of the control acupuncture group and 9.1% of the relaxation group. The patients who received acupuncture also had longer periods of sustained abstinence compared to the other participants. Acupuncture when used in combination with other therapies may be an innovative, and effective way to treat cocaine addiction.

Avants KS, Margolin A, Holford TR, Kosten TR: A randomized controlled trial of auricular acupuncture for cocaine dependence. Archives of Internal Medicine, 160(15):2305-12. 2000.

New Medications and Dosing Requirements Offer Options. As researchers and treatment providers search for ways to treat more patients with drug problems, some promising new medications are offering hope. Researchers have found that the dose requirement of a safe and effective drug for heroin addiction, buprenorphine, can be safely increased so that it can be administered on a less regular basis than other opioid dependence treatments. The study provides support for the safety and feasibility of administering buprenorphine twice a week in a medically supervised environment. Given that the frequent daily dosing schedule currently required by federal regulations has been shown to be a major obstacle for many patients in need of treatment, the implementation of this finding into practice may

attract more patients to treatment. It may also increase the ability of clinics to serve more patients without a proportional increase in staff.

Petry NM, Bickel WK, Badger GJ: A comparison of four buprenorphine dosing regimens in the treatment of opioid dependence. Clinical Pharmacology and Therapeutics, 66(3):306-14. 1999.

Medication Found Effective Against Toxic Effects of Cocaine in Mice. Cocaine abuse can lead to seizures, long-term neurological and psychiatric impairment, and death. Seizures can occur after the recreational use of relatively low doses of cocaine, as well as following an overdose. In a recent study researchers have discovered that chlormethiazole, acting through the brain's GABA system, protects against cocaine-induced seizures in mice. These results suggest that chlormethiazole may be an effective treatment for the life-threatening complications of cocaine abuse.

Gasior M, Ungard JT, Witkin JM: Chlormethiazole: effectiveness against toxic effects of cocaine in mice. Journal of Pharmacology and Experimental Therapeutics, 295(1):153-61. 2000.

Altering Brain Serotonin Levels May Reduce Aggressive and Impulsive Behaviors. Excessive impulsive and aggressive behaviors are seen in a number of psychopathological conditions including conduct disorder, attention deficit hyperactivity disorder, and substance abuse disorder. Laboratory studies have shown that aggression and impulsivity can be affected predictably through the manipulation of brain serotonin levels. Building on this research, investigators administered doses of d,l-fenfluramine, a substance which causes the release of serotonin, to a group of men diagnosed with conduct disorder. Increasing doses of d,l-fenfluramine produced decreases in aggressive and impulsive behaviors in the patients. This study indicates that the possible biological mechanisms for reduction of aggression and impulsive behaviors may involve the regulation of serotonin, which may also prove effective in treating substance abuse disorders.

Cherek DR, Lane SD: Effects of d,l-fenfluramine on aggressive and impulsive responding in adult males with a history of conduct disorder. Psychopharmacology, 146(4):473-81. 1999.

Better Understanding of Pain Mechanisms May Yield New Medications. Available pain medications, while essential for the management of pain, can produce undesirable side-effects as well as tolerance and dependence. Morphine and other similar pain medications act by attaching to specific sites or receptors on neurons that are known as opiate receptors. Recently, using advanced genetic techniques, researchers have identified several new subtypes of opiate receptors that may also be involved in morphine analgesia. These newly discovered subtypes appear to play a role in the production of analgesia that is different from previously identified opiate receptor subtypes. By targeting these new receptor subtypes, it may be possible to develop new medications for treating pain that have fewer or no undesirable side effects, including the development of tolerance and dependence.

Pan YX, Xu J, Bolan E, et al: Isolation and expression of a novel alternatively spliced mu opioid receptor isoform, MOR-1F. FEBS Letters, 466(2-3):337-40. 2000.

Zhu Y, King MA, Schuller AGP, et al: Retention of supraspinal delta-like analgesia and loss of morphine tolerance in delta opioid receptor knockout mice. Neuron, 24(1):243-52. 1999.

Testing with Magnets Speeds Recovery from Stroke or Brain Damage. Magnets, components of contemporary imaging machines, are viewed with optimism within the complementary and alternative medical community as effective healing tools for epilepsy, back pain, and other conditions. Previously, it was shown that powerful magnetic pulses affect the brain's motor cortex by mimicking simple nerve impulses, like those responsible for flexing individual fingers of the hand. Research using this method has shown that there is a short-term memory for movement, a first step in learning motor skills. Recent studies have identified several steps in the biochemical pathway of this process. Learning the method for magnetic stimulation of the brain provides valuable information that could lead to better recovery of function after stroke, perhaps revolutionizing the rehabilitation process.

Bütefisch CM, Davis BC, Wise SP, et al: Mechanisms of use-dependent plasticity in the human motor cortex. Proceedings of the National Academy of Sciences, 97(7):3661-65. 2000.

New Evidence Confirms Effect of Acupuncture on Morphine Withdrawal, Identifies Mechanism. Previous research indicated that electroacupuncture (EA) and transcutaneous electrical nerve stimulation (TENS) can be used to treat the effects of morphine withdrawal in both rat models and humans. However, the mechanism underlying this effect has remained unclear. Now, a recent study found that administering EA to rats increases the concentration of *dynorphin*, a regulatory protein of the central nervous system. Scientists report that the increased presence of *dynorphin* appears responsible for the analgesic effect and diminished withdrawal symptoms found in morphine-dependent rats. These results show the link between CAM therapy and specific biochemical events at the cellular level, and elucidate EA's potential therapeutic benefit in treating opiate addiction.

Wu LZ, Cui CL, Tian JB, Han JS: Suppression of morphine withdrawal by electroacupunture in rats: dynorphin and k-opioid receptor implicated. Brain Research, 851(1-2):290-96. 1999.

Natural Compound May Protect the Brain from the Effects of Aging. The Hemsley plant (*Cynanchum wilfordii*) is one of many herbs used in traditional Asian medical practices. Originally employed as a component of tonics or a diuretic, its potential for treating dementia and memory loss were recently evaluated. Studies showed that cyandione A, a compound extracted from the plant, protected cultured nerve cells from harmful substances that accumulate in aging or diseased brains. These findings demonstrate that in addition to *Ginkgo biloba*, other botanicals may provide an important source of compounds worthy of study for their neuroprotective properties.

Lee MK, Yeo H, Kim J, Markelonis GJ, Oh TH, Kim YC: Cynandione A from *Cynanchum wilfordii* protects cultured cortical neurons from toxicity induced by H_2O_2, L-glutamate, and kainate. Journal of Neuroscience Research, 59(2):259-64. 2000.

Bone Marrow Transplant May Have Long-Term Effect in Treating X-ALD. The childhood-onset form of X-linked adrenoleukodystrophy (X-ALD), a degenerative disorder of the central nervous system, leads to a vegetative state and death within 3-5 years once clinical symptoms are detectable. Researchers tested whether bone marrow transplantation can, over an extended period of time, halt the progressive neurological deterioration found in these X-ALD patients. They found that 5-10 years after treatment, patients receiving the bone marrow transplants had no further decline in verbal or motor performance, and a few showed an improvement in, or a complete reversal of symptoms. These findings demonstrate that this procedure can be effective, with long-lasting results, when it is done at an early stage of the disease.

Shapiro E, Krivit W, Lockman L, et al: Long-term effect of bone marrow transplantation for childhood-onset cerebral X-linked adrenoleukodystrophy. The Lancet, 356(9231):713-8. 2000.

Combination Therapy Improves Immune Function in HIV-1-Infected Children. Major strides have been made in combating HIV in adults using a multidrug regimen that includes a potent class of anti-HIV drugs known as protease inhibitors. Until recently, however, it was unknown if this approach was safe and effective for HIV-infected children. Thus, researchers evaluated several different drug regimens in children: 1) a 3-drug regimen which included the protease inhibitor, ritonavir, 2) a 2-drug regimen that included ritonavir and 3) a 2-drug regimen that *did not* include ritonavir. They found that the 3-drug regimen was well tolerated by the children, and was more effective in eliminating detectable levels of the AIDS virus than either of the 2-drug regimens. This trial underscores the feasibility, need, and importance of providing data about drugs to treat children not only to ensure their safety, but to ensure that children obtain the most effective therapies available.

Nachman SA, Stanley K, Yogev R, et al: Nucleoside analogs plus ritonavir in stable antiretroviral-experienced HIV-infected children: a randomized controlled trial. The Journal of the American Medical Association, 283(1-4):492-8. 2000.

Development of New Anti-Cancer Agents. Taxoids, a family of anti-tumor drugs that include Taxol, approved for the treatment of ovarian, breast, and lung cancer and Taxotere, approved for the treatment of breast cancer, act by a unique mechanism leading to interruption of the cell cycle and induction of cell death. While these drugs are potent and clinically exciting anti-tumor agents, their use is often accompanied by troublesome side effects as well as by the occurrence of multi-drug resistance (MDR). Several new, highly active "second-generation" taxoids have been developed and, upon testing in human breast cell carcinoma cell lines, were found to be at least as active as the approved drugs and, most significantly, equally active in MDR human breast carcinoma cell lines. One such drug, currently in

Phase 1 clinical trials, is taken orally and would be an important breakthrough in the administration of cancer drugs previously given only intravenously.

Ojima I, Wang T, Miller ML, et al: Synthesis and structure-activity relationships of second-generation taxoids. Bioorganic and Medicinal Chemistry Letters, 9(24):3423-8. 1999.

Targeting the Body's HIV Sanctuary Sites. HIV protease inhibitors are drugs that have proven remarkably effective in treating HIV-1 infection, a predominant strain of the AIDS virus that is seen worldwide. However, in some areas of the body such as the brain and testes, tissues appear to be protected from exposure to HIV protease inhibitors due to drug entry that is limited by P-glycoprotein, a membrane efflux transporter located in the capillary lining that extrudes the drug. Intravenous administration of selective potent inhibitors that target P-glycoprotein have resulted in beneficial higher drug levels in both the brain and testes of mice due to increased tissue penetration. These exciting studies involving the manipulation of a cellular efflux transporter may be widely applicable to different classes of drugs, including anticancer agents.

Choo EF, Leake B, Wandel C, et al: Pharmacological inhibition of P-glycoprotein transport enhances the distribution of HIV-1 protease inhibitors into brain and testes. Drug Metabolism and Disposition, 28(6):655-60. 2000.

New Class of Antibiotics. Deoxystreptamine-based aminoglycosides are a clinically important class of antibiotics that are effective against a broad range of microorganisms. Unfortunately, the high toxicity and rapid emergence of high-level aminoglycoside resistance have severely limited the usefulness of this class of antibiotics. Numerous resistance mechanisms to these drugs have been identified and the primary causes of high level resistance have been determined. A family of synthetic aminoglycosides that show great promise as antibiotics has been developed, one of which is active against a strain of bacteria that is aminoglycoside-resistant. Even more exciting was the unexpected finding that not only is this promising drug lead an antibiotic, but it also inhibits one of the enzymes that is responsible for antibiotic resistance.

Sucheck SJ, Wong AL, Koeler KM, et al: Design of bifunctional antibiotics that target Bacterial rRNA and inhibit resistance-causing enzymes. Journal of American Chemical Society, 122(21):5230-1. 2000.

Scaffolding for Nerve Cell Growth. Mammalian nerve cells are extremely finicky with respect to growth conditions. Scaffold material, constructed from peptides specifically bioengineered to be self-assembling, has been developed which allows nerve cells to attach, grow, and form active synaptic interactions (i.e., transmissions of an impulse from one nerve to another by either electrical or chemical means). While many types of scaffolding are being developed for a variety of tissues, finding ones suitable for nerve tissue has been difficult. Importantly, the scaffolding material is biologically inert, meaning it does not induce any inflammatory or immune responses when introduced into a host. The fact that the scaffolding has supported the development of active interactions between nerve cells grown

in culture points to the potential that the material may be used to help repair damaged neurological tissue.

Holmes TC, de Lacalle S, Su X, Liu G, Rich A, Zhang S: Extensive neurite outgrowth and active synapse sormation on self-sssembling peptide scaffolds. Proceedings of the National Academy of Sciences, 97(12):6728-33. 2000.

New Advice for Inhaled Corticosteroid Use in COPD. Chronic obstructive pulmonary disease (COPD) is a disorder that is a result of accelerated decline in lung function. It is thought to be caused by inflammatory changes which can be initiated by cigarette smoke. Corticosteroids are anti-inflammatory agents that are widely prescribed for COPD. Researchers recently concluded that their use does not alter the rate of decline of lung function in people with mild to moderate COPD, but does have a modest benefit in terms of airway reactivity, respiratory symptoms, and health care use for respiratory problems. This suggests that inhaled corticosteroids should be used only for reducing symptoms rather than for trying to modify the long-term course of the disease.

Lung Health Research Group. Effect of Inhaled Triamcinolone on the Decline in Pulmonary Function on Chronic Obstructive Pulmonary Disease. New England Journal of Medicine in press.

Umbilical Cord Blood Transplants Continue to Show Promise for Pediatric Patients. Graft versus host disease (GVHD) is a serious problem for recipients of bone marrow transplants, even when doctors are able to find donor cells that appear to be compatible with the patient. Recently, an international team of scientists reported that children needing stem cell transplants are at less risk of developing GVHD if they receive their brother's or sister's umbilical cord blood (UCB) instead of bone marrow donated by a sibling. When combined with evidence that UCB donors need not match the recipients as closely as bone marrow donors do, this finding suggests that transplantation of unrelated UCB may be useful for most children who need stem cell transplants, even those with hard-to-match tissue types.

Rocha V, Wagner JE, Sobocinski KA, et al: Graft-versus-host disease in children who have received a cord-blood or bone marrow transplant from an HLA-identical sibling. The New England Journal of Medicine, 342(25):1846-54. 2000.

Cause of Kidney Failure in Diabetic Mice. Diabetes is the most common cause of end-stage kidney disease. Researchers report that, in mice that have diabetes, kidney failure is caused by overproduction of a protein called transforming growth factor b (TGF-ß). TGF-b causes scar tissue inside the kidneys, which eventually interferes with kidney function. Key to this finding is that, when the protein is neutralized with an antibody against it, the mice do not develop kidney failure. This is the first proof-of-concept study showing that TGF-b may cause end-stage kidney disease. Drug treatments for kidney failure, targeted to inhibition of TGF-b can now be pursued. This advance is relevant to both type 1 and type 2 diabetes, since kidney complications often occur in both forms of the disease.

Ziyadeh FN, Hoffman BB, Han DC, et al: Long-term prevention of renal insufficiency, excess matrix gene expression, and glomerular mesangial matrix expansion by treatment with monoclonal antitransforming growth factor-beta antibody in db/db diabetic mice. Proceedings of National Academy of Sciences, 97(14):8015-20. 2000.

Nitric Oxide: Possible Treatment Approach for Sickle Cell Anemia. Hemoglobin, also known as globin, is the molecule in red blood cells responsible for transporting oxygen throughout the body. Individuals with sickle cell anemia have a mutation in one of the globin genes that alters the structure of the molecule. This structural change causes distortion of the red blood cell, producing the "sickle" shape for which the disease is named. Because of their shape, these cells can block the flow of blood in the small vessels resulting in severe pain and destruction of tissue and organs. These cells also have a lower affinity for oxygen than normal cells. Treatment with nitric oxide (NO) reportedly increases oxygen affinity of sickle cells. It has been proposed that NO may cause small blood vessels to dilate, which would allow easier passage of the sickle shaped cells through small vessels and into surrounding tissue. In one study, patients with sickle cell anemia were treated by inhaling nitric oxide. Although this treatment did increase overall oxygen affinity, the researchers observed a dose-dependent increase in nitric oxide being transported by hemoglobin. These results suggest that new treatment strategies should be considered that utilize nitric oxide transport to improve blood flow through small vessels and into tissue.

Gladwin MT, Schechter AN, Shelhamer JH, et al: Inhaled nitric oxide augments nitric oxide transport on sickle cell hemoglobin without affecting oxygen affinity. Journal of Clinical Investigation, 104(7):937-45. 1999.

Liver Cells Derived from Bone Marrow in Humans. Stem cells have been defined by their unique ability to both replenish their stores by cell division and to differentiate into specialized cells, such as liver, muscle, blood and nerve cells. Mature cells, however, are assumed to have only the capability of producing cells of their own type. The regeneration of the liver after massive resection or severe injury has been postulated to be due in part to differentiation of resident stem cells into mature hepatocytes and bile duct cells. However, stem cells have not been conclusively identified in adult human liver. It has now been shown that human bone marrow cells can migrate to the liver and differentiate into mature functional liver cells. Analyzing tissue samples obtained from women who had received a bone marrow transplant from a male donor, researchers identified liver cells containing a Y chromosome. Similarly, they identified Y chromosome containing liver cells in male subjects who had received a liver transplant from a woman. These findings demonstrate that mature liver cells can be derived from circulating cells, most likely of bone marrow origin. This research has major implications for management of fulminant hepatic failure and genetic liver diseases by hepatocyte transplantation and gene therapy.

Theise ND, Nimmakayalu M, Gardner R, et al: Liver from bone marrow in humans. Hepatology, 32(1):11-16. 2000.

Correction of Membrane Lipid Imbalance Restores Phenotypic Expression in CFTR$^{-/-}$ Mice. Thirty thousand Americans have cystic fibrosis, a lethal genetic disease which limits their lifespan to approximately 30 years. These individuals are unable to synthesize the protein coded for by the cystic fibrosis (CF) gene. Absence of this protein affects the mucus glands of the body resulting in thick, viscous secretions leading to serious respiratory and digestive problems. Mice lacking the CF gene were used to investigate whether a previously reported deficiency in the fatty acid metabolism of CF patients exists only in organs affected by the disease and whether it plays a role in the phenotypic expression of CF. A significant membrane fatty acid (or lipid) imbalance was observed in organs in which pathogenesis is expressed; the lungs, pancreas and ileum. This imbalance is characterized by an increase in phospholipid bound arachidonic acid (AA: omega-3 fatty acid) and a decrease in docosahexaenoic acid (DHA: omega-6 fatty acid). From these data, researchers suggested that membrane lipid metabolism is altered by the lack of the CF gene and may secondarily lead to the clinical symptoms and signs of this disease. When the CF mice were fed increased amounts of DHA, the metabolic lipid imbalance was corrected and phenotypic expression and complications of CF were reversed. These important findings have expanded our understanding of the pathogenesis of CF and will have a profound impact on the development of new therapies for treatment of this disease.

Freedman SD, Katz MH, Parker EM, Laposata M, Urman MY, Alvarez JG: A membrane lipid imbalance plays a role in the phenotypic expression of cystic fibrosis in CFTR(-/-) mice. Proceedings of National Academy of Sciences, 96(24):13995-14000. 1999.

Improved Treatment for Cholera. Each year three million children die worldwide from cholera and other acute, infectious diarrheal diseases. These deaths are largely due to severe dehydration from the massive loss of salt and water associated with infectious diarrhea. Currently, rehydration therapy using oral glucose solutions, which increase absorption of salt and water by the small intestine, is the standard method of treatment. Although this therapy corrects the dehydration caused by cholera, it does not reduce the diarrhea itself. Researchers seeking to improve diarrheal treatment of cholera administered amylase-resistant starch in combination with rehydration therapy. This approach is based on physiological studies of the colon that demonstrated that short-chain fatty acids produced by bacteria in the colon provide nutrients to colonocytes and stimulate absorption of water and electrolytes. Amylase-resistant starch is not absorbed in the small intestine but does provide nutrients for bacteria when it reaches the colon. The bacteria then produce more short chain fatty acids which might stimulate increased water and electrolyte absorption. This theory was tested in a randomized clinical trial conducted in India which showed that the addition of amylase-resistant starch to standard rehydration therapy significantly decreased the duration and volume of diarrhea in patients with cholera. This new therapy may also be applicable to management of other diarrheal illnesses and provides improved means of managing cholera that are applicable to undeveloped areas of the world.

Ramakrishna BS, Venkataraman S, Srinivasan P, Dash P, Young GP, Binder HJ: Amylase-resistant starch plus oral rehydration solution for cholera. The New England Journal of Medicine, 342(5):308-13. 2000.

Genetic Correction of Sickle Cell Disease. Sickle cell disease (SCD) is caused by a genetic abnormality in the adult form of hemoglobin that causes red blood cells to take on a "sickling" shape. Patients with SCD who also produce the fetal form of hemoglobin generally have reduced clinical evidence of SCD. Using a mouse model of SCD and a second mouse model that expresses the human fetal hemoglobin gene, researchers mated the two types of animals to produce offspring that showed considerable improvement in the SCD of the former model. Direct therapeutic effects of fetal hemoglobin were demonstrated. The mouse models may be used for quantitative assessment of the contribution of fetal hemoglobin to the inhibition of sickling and for developing new gene therapy approaches for SCD.

Blouin MJ, Beauchemin H, Wright A, et al: Genetic correction of sickle cell disease: insights using transgenic mouse models. Nature Medicine, 6(2):177-82. 2000.

Agent Found Effective in Treating HIV Lipodystrophy Syndrome. Emerging reports describe metabolic abnormalities and body composition changes in HIV-infected individuals using antiretroviral therapies, including abnormal glucose metabolism and distribution of fat, referred to as lipodystrophy. These patients then have an increased propensity to insulin resistance, diabetes mellitus, and heart disease – effects that may impact on the long-term prognosis of patients whose compromised life expectancies have been significantly extended due to decreased viral load. Researchers hypothesized that treatment with an insulin-sensitizing agent called metformin would improve insulin resistance and risk of developing heart disease in these patients. Over a period of three-months, they studied 26 HIV-infected, non-diabetic patients with lipodystrophy and abnormal blood glucose levels. Fourteen patients received treatment with metformin. Preliminary data demonstrated that low-dosage treatment with this agent reduced insulin resistance and improved weight and blood pressure. The medication was well tolerated and did not interfere with the patients' antiretroviral regimen. Though further studies are needed to determine the long-term benefits of insulin-sensitizing agents such as metformin, therapies can now be developed that improve insulin resistance and fat redistribution, yet permit patients to be maintained on their other medications.

Hadigan C, Corcoran C, Basgoz N, Davis B, Sax P, Grinspoon S: Metformin in the treatment of HIV lipodystrophy syndrome: a randomized controlled trial. The Journal of American Medical Association, 284(4):472-7. 2000.

Successful Treatment of Kaposi's Sarcoma with Thalidomide. Kaposi's sarcoma (KS), the most common cancer in patients infected with the human immunodeficiency virus (HIV), depends heavily on the development of tiny blood vessels (angiogenesis) to feed the tumors. In a recent clinical study, KS patients were treated with thalidomide, a drug with known anti-angiogenic properties. Tumors shrunk in nearly half of the patients; it is likely that thalidomide caused the shrinkage through its anti-angiogenic activity. While additional studies are needed, this study, in addition to suggesting an effective new treatment for KS, is one of the first studies to demonstrate that an anti-angiogenic drug can cause remissions in an established tumor.

Little RF, Wyvill KM, Pluda JM, Welles L, et al. Activity of thalidomide in AIDS-related Kaposi's sarcoma. Journal of Clinical Oncology, 18(13):2593-602. 2000.

Managing Menopausal Symptoms in Breast Cancer Survivors. Menopausal symptoms (e.g., hot flashes, vaginal dryness, and stress urinary incontinence) are very common in breast cancer survivors and cannot be managed with standard estrogen replacement therapy in these patients. Researchers explored the use of a comprehensive menopausal assessment intervention program, focusing on symptom assessment, education, counseling and, as appropriate, specific pharmacologic and behavioral interventions, to assist breast cancer survivors in managing their menopausal symptoms. Patients who received the intervention experienced improvement in menopausal symptoms and sexual functioning. These findings will have an important impact on the quality of life of breast cancer survivors, whose numbers are expected to increase in the coming years.

Ganz PA, Greendale PA, Petersen L, Zibecchi L, Kahn B, Belin TR: Managing menopausal symptoms in breast cancer survivors: Results of a randomized controlled trial. Journal of the National Cancer Institute, 92(13):1054-64. 2000.

Progress with Antibodies as Anticancer Agents. Monoclonal antibodies are laboratory- produced proteins that can locate and bind to specific proteins expressed by cancer cells. NIH-supported researchers are exploring the use of a monoclonal antibody called C225 in cancer treatment. C225 binds to epidermal growth factor receptors, which are proteins that are frequently overproduced in cancer cells, and inhibits the cancer cells' growth. In a recent series of Phase I studies, C225 showed activity against epithelial tumors and head and neck cancer when given alone or in combination with standard treatments, and was well tolerated by patients. C225 is also under study in combination with other treatments against head and neck and pancreatic cancers.

Baselga J, Pfister D, Cooper MR, et al: Phase I studies of anti–epidermal growth factor receptor chimeric antibody C225 alone and in combination with cisplatin. Journal of Clinical Oncology, 18(4):904-14. 2000.

Treatment of Leukemia and Lymphoma with Recombinant Immunotoxins. Immunotoxins are composed of antibodies, or proteins that bind to specific foreign substances within the body, linked to a toxin. Some immunotoxins can be targeted to cancer cells; the antibody latches onto the cancer cell, and the toxin kills it. The recombinant immunotoxin LMB-2 has shown promise against several types of leukemia and lymphoma in Phase I studies, and will enter Phase II studies late in 2000. Meanwhile, researchers are developing methods to decrease the side effects of treatment with LMB.

Kreitman RJ, Wilson WH, Robbins D, et al: Responses in refractory hairy cell leukemia to a recombinant immunotoxin. Blood, 94(10):3340-48. 1999.

Kreitman RJ, Wilson WH, White JD, et al: Phase I trial of recombinant immunotoxin anti-Tac(Fv)-PE38 (LMB-2) in patients with hematologic malignancies. Journal of Clinical Oncology, 18(8):1622-36. 2000.

Onda M, Kreitman RJ, Vasmatzis G, et al: Reduction of the nonspecific animal toxicity of antiTac(Fv)-PE38 by mutations in the framework regions of the Fv which lower the isoelectric point. Journal of Immunology, 163(11):6072-7. 1999.

Tsutsumi Y, Onda M, Nagata S, et al: Site-specific PEGylation of recombinant immunotoxin antiTac(Fv)-PE38 (LMB-2) improves antitumor activity and reduces animal toxicity and immunogenicity. Proceedings of the National Academy of Sciences, 97(15):8548-53. 2000.

A New Approach for Treating Menstrual Epilepsy. For over 100 years it has been known that women with epilepsy often experience a greater number of seizures at the time of menstruation – called "catamenial epilepsy." Researchers have found that the female reproductive hormone progesterone is converted in the brain to allopregnanolone, a natural steroid with protective activity against seizures. Just before menstruation there is a drop in the brain content of allopregnanolone, leading to an increase in seizure susceptibility. In animal studies, researchers found that a synthetic analog of allopregnanolone can reverse the increase in seizure susceptibility associated with a fall in brain allopregnanolone, which points the way toward treatments to prevent the menstrual seizures that impair the quality of life of many women with epilepsy.

Kokate TG, Banks MK, Magee T, Yamaguchi S, Rogawski MA: Finasteride, a 5a-reductase inhibitor, blocks the anticonvulsant activity of progesterone in mice. Journal of Pharmacology & Experimental Therapeutics, 288(2):679-84. 1999.

Reddy DS, Rogawski MA: Enhanced anticonvulsant activity of ganaxolone after neurosteroid withdrawal in a rat model of catamenial epilepsy. Journal of Pharmacology and Experimental Therapeutics, 294(3):909-15. 2000.

Drugs for Fabry Disease. People with Fabry disease inherit a deficiency of the enzyme alph-galactosidase A. This leads to buildup of large fatty molecules that this enzyme normally breaks down, impairing circulation, damaging kidneys, heart, and brain, leading to premature death. Efforts to treat Fabry disease by replacing the missing enzyme are underway and early results from clinical trials are promising. However, enzyme replacement therapy is extremely expensive and may not fully counteract the problems this disorder causes in the brain. Scientists have now developed a drug that helps mice with Fabry disease by blocking the synthesis of substances the missing enzyme normally helps break down. "Storage diseases," in which a defective enzyme leads to harmful accumulations, are common and often severely affect the brain. This drug treatment strategy, with different specific blockers, may ultimately apply to many of these disorders.

Abe A, Gregory S, Lee L, et al: Reduction of globotriaosyleramide in fabry disease mice by substrate deprivation. Journal of Clinical Investigation, 105(11):1563-71. 2000.

Minimizing "Secondary Damage" Following Spinal Cord Injury. Finding a cure for spinal cord injury is a dauntingly complex problem. One aspect is minimizing the "secondary damage" that continues in the hours and days following initial trauma. Several years ago NIH supported trials proved

that the steroid drug methylprednisolone can help in this respect, and this treatment is now standard practice for acute spinal cord injury. In separate efforts to improve on this strategy scientists this year demonstrated in rats that injection following spinal cord injury of a natural growth factor FGF2 (basic fibroblast growth factor), of TTX (a toxin that blocks electrical activity), of DNQX (a drug that blocks certain receptors for the neurotransmitter glutamate), or of interleukin-10 (an immune system signaling molecule) can reduce spinal cord damage following injury. Most scientists believe that a combination of approaches to minimize damage and to foster regeneration will be the best strategy for reducing the enormous burden imposed by spinal cord injury.

Teng YD, Mocchetti I, Taveira-DaSilva AM, Gillis RA, Wrathall JR: Basic fibroblast growth factor increases long-term survival of spinal motor neurons and improves respiratory function after experimental spinal cord injury. The Journal of Neuroscience, 19(16):7037-47. 1999.

Rosenberg LJ, Teng YD, Wrathall JR: Effects of the sodium channel blocker tetrodotoxin on acute white matter pathology after experimental contusive spinal cord injury. The Journal of Neuroscience, 19(14):6122-33. 1999.

Rosenberg LJ, Teng YD, Wrathall JR: 2,3-Dihydroxy-6-nitro-7-sulfamoyl-benzo(f)quinoxaline reduces glial loss and acute white matter pathology after experimental spinal cord contusion. The Journal of Neuroscience, 19(1):464-75. 1999.

Bethea JR, Nagashima H, Acosta MC, et al: Systemically administered interleukin-10 reduces tumor necrosis factor-alpha production and significantly improves functional recovery following traumatic spinal cord injury in rats. Journal of Neurotrauma, 16(10):851-63. 1999.

Brewer KL, Bethea JR, Yezierski RP: Neuroprotective effects of interleukin-10 following excitotoxic spinal cord injury. Experimental Neurology, 159(2):484-93. 1999.

Tachyphylaxis May Not Exist! Tachyphylaxis is thought to occur in treating skin disease when there is a rapidly decreasing response to a physiologically active agent after administration of a few doses. This phenomenon is commonly attributed to the skin getting used to the product, to the onset of total tolerance for the drug. Investigators looked at the treatment of plaque psoriasis with topical corticosteroids over a period of 12 weeks, well beyond the amount of time during which tachyphylaxis would be expected to occur. The researchers concluded that what dermatologists in clinical practice call tachyphylaxis is simply the failure of topical treatment to completely clear the psoriasis after an initial period of improvement. Long-term use of topical corticosteroids may result in some adverse effects, particularly thinning of the skin and visible dilated blood vessels. This study points out that for diseases like psoriasis, for which there is no cure, physicians and the public must be aware of the need to combine therapies and/or rotate therapies once maximum clinical benefit is achieved short of resolution of the disease.

Miller J, Roling D, Margolis D, Guzzo C: Failure to demonstrate therapeutic tachyphylaxis to topically applied steroids in patients with psoriasis. Journal of the American Academy of Dermatology, 41(4):546-9. 1999.

Interleukin-12 May Be Useful in Treatment of Cutaneous T-Cell Lymphoma. Cutaneous T-cell lymphoma (CTCL) is a type of skin cancer associated with profound defects in cell-mediated immunity. Cell-mediated immunity is supported by a variety of chemical produced by the body. One of these agents, interleukin-12 (IL-12), has shown to be reduced dramatically in patients with CTCL. Investigators initiated a clinical trail with recombinant human IL-12 in patients with CTCL. The results suggest that IL-12 may be a potent and well-tolerated therapeutic agent for CTCL. Although CTCL is a relatively rare disease, it is a disease with no known cure. Use of IL-12 could provide a new approach with low toxicity for patients with an otherwise incurable and potentially fatal disease.

Rook AH, Wood GS, Yoo EK, Elenitsas R, Kao DM, Sherman ML, et al: Interleukin-12 therapy of cutaneous T-cell lymphoma induces lesion regression and cytotoxic T-cell responses. Blood, 94(3):902-8. 1999.

Understanding the Immunologic Basis of Psoriasis Leads to Therapeutic Interventions. Psoriasis is a chronic skin disease that can be extensive and disabling. In the last 5-10 years, it has been recognized that the underlying cause is an abnormality in control of immune cell-stimulated inflammation that results in the clinical disease. Using an animal model in which human psoriasis was grafted onto immunosuppressed mice, scientists investigated how approved psoriasis treatment drugs (Dovoenex and cyclosporine A) work in suppressing psoriasis. They discovered that the agents were active via an immunomodulatory mechanism working through T cells. Understanding the mechanism of action of approved drugs can lead to design of more effective and less toxic drugs.

Dam TN, Kang S, Nickoloff BJ, Voorhees JJ: 1-alpha, 25-dihydroxycholecalciferol and cyclosporine suppress induction and promote resolution of psoriasis in human skin grafts transplanted on to SCID mice. Journal of Investigative Dermatology, 113(6):1082-9. 1999.

Treatment for Lyme Disease Does Not Appear to Result in Long-Term Neurologic and Musculoskeletal Problems. Lyme disease is a multisystem disorder, affecting the skin, nervous system, heart, and joints. It is caused by tick-borne infection with *Borrelia burgdorferi*. Previous followup studies of patients with Lyme disease suggest that some may suffer from long-term neurologic and musculoskeletal complications when they had the infection for a long period before receiving treatment. Researchers conducted a population-based, retrospective study of individuals who had a history of Lyme disease and controls. Examination of these individuals 6 years after infection failed to reveal increased neurologic or musculoskeletal complications in those treated for Lyme disease over those who never had Lyme disease. People treated for Lyme disease in the late 1980s do not experience more long-term neurologic or musculoskeletal problems than those who have never had the disease.

Shadick NA, Phillips CB, Sangha O, Logigian EL: et al: Musculoskeletal and neurologic outcomes in patients with previously treated Lyme disease. Annals of Internal Medicine, 131(12):919-26. 1999.

Endotoxins May Play a Role in Orthopaedic Implant Loosening. More than 400,000 total hip and knee replacements are performed each year in the United States for end-stage arthritis. Although generally successful at the outset, these procedures can eventually be complicated by aseptic loosening and osteolysis. Osteolysis is the disappearance of bone around the implant in response to the presence of microscopic wear particles, which are believed to incite an inflammatory response. Investigators hypothesized that biological responses induced by wear particles may be due to adherent endotoxins, a major component of cell walls. Recent research suggests that standard techniques used to purify implant surfaces vary in their effectiveness in removing adherent endotoxins and may account for disparate results in studies comparing the magnitude of biological responses induced by different types of materials. If it is proven that endotoxins do, indeed, play a role in orthopaedic implant loosening, it may be prudent for implant manufacturers to remove adherent endotoxins from implants prior to surgery.

Ragaab AA, Van De Motter R, Lavish SA, et al: Measurement and removal of adherent endotoxin from titanium particles and implant surfaces. Journal of Orthopedic Research, 17(6):803-9. 1999.

Development of Sulfamethoxazole Resistant Pneumocystis Carinii in Patients with HIV Infection. Pneumocystis pneumonia, the most common life threatening AIDS associated opportunistic infection, and a frequent cause of life thretening pneumonia in patients with cancer and transplants, has been thought to be universally sensitive to the drug sulfamethoxazole, the primary agent used for therapy of acute pneumonia and for prevention. Investigators identified two patients who had mutations in the target enzyme in the pneumocystis organism for sulfamethoxazole, but not for trimethoprim. Data from other sources suggests that these mutations are more common among patients who have been exposed to sulfamethoxazole, and among patients who fail acute therapy. The emergence of sulfamethoxazole resistance is an ominous development that is being monitored in prospective studies; more efforts to develop alternative drugs are needed.

Ma L, Boria L, Masur H, Kovacs JA: Pneumocystis carinii dihydropteroate synthase but not dihdrofolate reductase gene mutations correlate with prior trimethoprim sulfamethoxazole or dapsone use. Journal of Infectious Diseases, 180(6):1969-78. 1999.

Minimally-invasive, Image Guided, Percutaneous Tumor Ablation with Radiofrequency Energy. Recent technological developments allow physicians to place small treatment needles or probes into organs, soft tissue, and solid tumors, and deposit radio wave energy in the tumor and rapidly, safely, effectively and predictably kill large tumors, without having to surgically remove the tumor. This research investigates the potential clinical applications of this new technology including patients with hereditary kidney cancer, liver cancer, adrenocortical carcinoma, or painful tumors with no other medical or surgical options.

Abraham J, Fojo T, Wood BJ: Radiofrequency ablation of metastatic lesions in adrenocortical cancer. Annals of Internal Medicine, 133(4):312-3. 2000.

New Imaging Technique for Following the Movements of Transplanted Brain Cells. Transplanted precursor (stem) cells have shown to be able to repair white matter disease in the brain and spinal cord, but their cellular migration and movements could, until today, only be evaluated using invasive and irreversible surgical procedures. Scientists have now developed a new, patented magnetic imaging technique that can track those cells in 3 dimensions, non-invasively, and repeatedly if necessary. This innovation has shown to be highly accurate in the mapping of newly formed white brain matter, and may guide future clinical therapies, in particular for the treatment of multiple sclerosis and spinal cord injury.

Bulte JW, Zhang S, van Gelderen P, et al: Neurotransplantation of magnetically labeled oligodendrocyte progenitors: magnetic resonance tracking of cell migration and myelination. Proceedings of the National Academy of Sciences, 96(26):15256-61. 1999.

Improved Dental and Orthopedic Implants. In the repair of the human body with prosthetics or artificial replacement parts, mechanical attachment to the body, or alternatively rejection by the body, occurs at the materials-biological systems interface. Therefore, effective manipulations of implantable material surfaces and /or creation of "smart" biocompatible materials for fabrication of artificial organs is a major challenge. For example, NIH-supported investigators have developed ways to improve Titanium (Ti)-based implants. Specifically, this involves the application of "bioactive" nanoparticle coatings on the surface of titanium implants to allow bonding of the implant to the adjoining bone. Development of bioactive coatings can improve the adhesion of Ti-based implants to the existing bone, resulting in significantly better implant lifetime than can be achieved with conventional materials in use today.

Gomez-Vega JM, Saiz E, Tomsia AP et al: Glass-Based Coatings for Titanium Implant Alloys. Journal of Biomedical Materials Research, 46(4) 549-59. 1999.

NSAIDs Suppress Alzheimer's Disease Pathology in an Animal Model. The possibility that an inflammatory response is involved in the pathogenesis of Alzheimer's disease (AD) has been suggested by epidemiological studies showing that the use of nonsteroidal anti-inflammatory drugs (NSAIDs) is associated with a 50% risk reduction in the development of AD. This study examined the impact of chronic oral administration of ibuprofen, an inhibitor of prostaglandin synthesis, in a transgenic mouse model with widespread age-related amyloid deposition, microglial activation, and dystrophic neurites. Several AD-like features were reduced. First, amyloid-beta deposition in plaques was lower in these animals. Second, glial markers were quantitatively lower, suggesting reduced inflammation. Third, treatment resulted in significant decreases in interleukin (IL)-1_, a potent pro-inflammatory agent. Over expression of IL-1_ is proposed to be a key element in the cascade of neurodegenerative events in AD. This study suggests that ibuprofen can significantly delay plaque deposition and associated pathology in a transgenic mouse model. Thus, long-term anti-inflammatory treatment can reduce

amyloid deposition when administered early in the disease course of these mice, suggesting that this treatment might also be effective in humans.

Lim GP, Yang F, Chu T, et al: Ibuprofen suppresses plaque pathology and inflammation in a mouse model for Alzheimer's disease. The Journal of Neuroscience, 20(15):5709-14. 2000.

Produce Produces Motor Benefits in Old Rats. Oxidative stress has been proposed to be a contributing factor to the aging process and to neurodegenerative diseases. Previous studies in old rats have shown that diets high in antioxidants are capable of delaying and perhaps even reversing the age-related onset of memory deficits. This study investigates whether antioxidants show similar positive benefits for motor coordination and motor learning. Old rats were fed diets supplemented with spinach, strawberries or blueberries. Spinach-fed animals reached a better level of performance than controls on a task where animals had to learn to make a motor response in order to receive a water reward. There was a trend for improvement in the blueberry-fed old rats. In addition, all three supplemented diets produced an improvement in the function of nerve cells in the cerebellum, a brain region important for the coordination of motor function and for motor learning. These findings suggest that vegetable and fruit supplements high in antioxidants have the potential for partially reversing age-related declines in motor function and motor learning.

Bickford PC, Gould T, Briederick L, et al: Antioxidant-rich diets improve cerebellar physiology and motor learning in aged rats. Brain Research, 866(1-2):211-7. 2000.

Gene Therapy with $VEGF_{121}$ Gene Transfer Stimulates Angiogenesis for Treatment of Peripheral Muscle Ischemia in Rats. One way to treat ischemic vascular disease is through application of angiogenic factors, which promote formation of new blood vessels, delivered through genetically altered viral vectors. Successful therapy of this sort would be expected to improve the bioenergetic characteristics of the affected tissue. Scientists successfully used NMR spectroscopy to measure high-energy phosphate metabolites in normal muscle distal to femoral artery resection in rats both before and after viral vector angiogenesis treatment. They found that viral vector vascular endothelial growth factor (VEGF), delivered to normal skeletal muscle several weeks previously, acted to normalize the pattern of high-energy phosphate response to muscle stimulation and recovery, indicating an increase in the rate of development of perfusing vessels. These results are the first to demonstrate the successful induction of angiogenesis in nonischemic skeletal muscle.

Gowdak LHW, Poliakova L, Wang X, et al: Adenovirus-mediated $VEGF_{121}$ gene transfer stimulates angiogenesis in normoperfused skeletal muscle and preserves tissue perfusion after induction of ischemia. Circulation, 102(5):565-71. 2000.

The Impact of Hormone Replacement Therapy on Iron Status in Women. Women's iron stores increase with age. When the body has more iron than it can bind to proteins such as hemoglobin,

ferritin, and transferrin, the resulting free iron may contribute to atherosclerosis and coronary heart disease. Hormone replacement therapy (HRT) is known to provide some vascular protection. Nurse researchers theorized that HRT's impact on iron status in postmenopausal might be one of these protective mechanisms. They studied 27 postmenopausal women who were using replacement hormones and 27 who were not. Diet and physical activity did not differ between the groups, but women taking hormones had lower serum ferritin and higher transferrin levels than women without HRT. Previous research has identified high ferritin and low transferrin as risk factors for heart disease. Thus, the lower serum ferritin of the women taking hormones may reflect lower, healthier levels of stored iron while their higher transferrin may suggest lower, safer levels of free iron. The investigators call for continued study of the mechanisms by which HRT protects postmenopausal women against heart disease.

Penckofer S, Schwertz D: Improved iron status parameters may be a benefit of hormone replacement therapy. Journal of Women's Health and Gender-Based Medicine, 9(2):141-51. 2000.

New Less Toxic Immunosuppressive Drug. An important class of immunosuppressive drugs blocks immune responses by preventing the interaction between the enzyme, calcineurin, and other proteins that are central to T cell functions. Unfortunately, standard immunosuppressive drugs are toxic and have serious side effects. Now, NIAID-funded scientists have designed a compound that acts far more selectively, yet efficiently inhibits T cell responses, *in vitro,* and may prove to be considerably less toxic. The development of new drugs based on this compound would represent a major advance in the treatment of graft rejection and allergic and autoimmune diseases.

Aramburu A, Yaffe M, Lopez-Rodríguez C, et al: Affinity-driven selection of an NFAT inhibitor more selective than cyclosporin A. Science, 285(5436):2129-32. 1999.

New Drug to Aid in Protection Against Bioterrorism. Although smallpox was eradicated in the 1970's, smallpox has re-emerged as a public health concern because of its potential use as a biowarfare agent. At present, no drugs exist to treat orthopoxviruses, the family of respiratory-transmitted viruses of which smallpox is a member. Recently, NIH-supported investigators tested the efficacy of a drug called cidofovir against these viruses. Cidofovir is currently licensed to treat an unrelated disease. The investigators, expanding upon prior findings by another research team, found that cidofovir inhibits a broad spectrum of orthopoxviruses *in vitro,* completely protects monkeypox-infected monkeys from signs of disease, and is highly effective in protecting and treating cowpox virus in mice. These findings suggest cidofovir may be effective treatment for smallpox infection.

Martinez MJ, Bray MP,Huggins JW: A mouse model of aerosol-transmitted orthopoxviral disease. Archives of Pathological Laboratory Medicine, 124(3):362-77. 2000.

Smee DF, Bailey KW, Wong M-H, Sidwell RW: Intranasal treatment of cowpox virus respiratory infections in mice with cidofovir. Antiviral Chemical Chemotherapy, 11(4):303-9. 2000.

Bray M, Martinez M, Smee DF, Kefauver D, Thompson E, Huggins JW: Cidofovir protects mice against lethal aerosol or intranasal cowpox virus challenge. Journal of Infectious Diseases, 181(1):10-19. 2000.

Promising Lead in Treatment of Hantavirus Pulmonary Syndrome. Hantavirus pulmonary syndrome (HPS) is the rodent-borne viral disease first identified in the 1993 "Four Corners" region of the southwestern United States. The fatality rate for HPS is roughly 50 percent, and, at the present time, no specific treatment is available. NIH-supported researchers recently established that patients whose blood contains a high level of hanatavirus neutralizing antibodies upon initial medical presentation are more like to survive than patients with lower levels of the antibody. These findings suggest that neutralizing immune plasma, extracted from survivors, might be a useful treatment for persons diagnosed HPS.

Bharadwaj M, Nofchissey R, Goade D, Koster F, Hjelle B: Humoral immune responses in the hantavirus cardiopulmonary syndrome. Journal of Infectious Diseases, 182(1):43-8. 2000.

The Many Faces of Nitric Oxide. A collaborative research project involving U.S. scientists and counterparts from Uruguay has demonstrated the dual roles of nitric oxide and oxygen-derived free radicals as critical mediators (both positive and negative) of tissue inflammatory reactions. With support from the Fogarty International Research Collaboration Award, the Uruguayan achievements include the discovery of nitric oxide-oxygen free radical interactions, the role of carbon dioxide in the modulation of inflammatory tissue injury and the protective, antioxidant actions of nitric oxide during inflammatory tissue injury. The results have important clinical implications for the management of inflammation and tissue injury during in such diverse areas as cardiopulmonary diseases and organ transplantation.

Alvarez B, Ferrer-Sueta G, Freeman BA Radi R: Kinetics of peroxynitrite reaction with amino acids and human serum albumin. Journal of Biological Chemistry, 274(2):842-8. 1999.

Eiserich JP, Estevez AG, Bamberg TV, et al: Microtubule dysfunction by posttranslational nitrotyrosination of alpha-tubulin: a nitric oxide-dependent mechanism of cellular injury. Proceedings of the National Academy of Sciences, 96(11):6365-70. 1999.

A New Approach to Dissecting the Cell Signaling Mechanism Involved in the Allergic Response. An allergen's bond to certain parts of certain cells of the immune system triggers a chain of events that may lead to allergic reactions or asthma. These reactions may be mild or life-threatening (such as anaphylactic shock). By studying the pathways from allergen binding to the allergic reaction, scientists from the U.S. and Mexico hope to understand the mechanism that control and interrupt these events. A recent publication described a new approach to dissecting the allergic-reaction-signaling-pathway using certain immune system cells called mast cells. The journal, Trends in Cell Biology, recognized the work as "headline making". The scientists' results pinpoint specific target enzymes that act as key players in transmitting the signal to release inflammatory substances that mediate the symptoms of an allergic response. Drugs may now be designed to block the action of these enzymes,

and therefore, the uncontrolled signal cascade leading to severe allergic reactions, asthma and anaphylactic shock.

Ortega E, Lara M, Lee I, et al: Lyn dissociation from phosphorylated Fc epsilon RI subunits: a new regulatory step in the Fc epsilon RI signaling cascade revealed by studies of Fc epsilon RI dimer signaling activity. Journal of Immunology, 162(1):176-85. 1999.

May R: Dimers are forever. Trends in Cell Biology, 9(6):214. 1999.

STORIES OF DISCOVERY

Nitric Oxide: An Air Pollutant or Life Saving Treatment for Newborn Infants?

Blue baby. At first glance, the term seems an innocent description of a character in a children's storybook. In reality, the term refers to newborns whose lungs cannot absorb enough oxygen for the babies to live. Recently, however, NIH-sponsored researchers pioneered a new treatment for the condition known as hypoxic respiratory failure. In a story typical of the twisting path of scientific discovery, the new treatment was developed from basic research on nitric oxide, a gas that is a major component of polluted air.

The story began in 1987, when researchers discovered that the gas caused the muscles controlling the lining of the heart and blood vessels to relax. At first, the scientific community was skeptical that a gas that is an air pollutant could perform such an important function. Later, research confirmed that nitric oxide was central to many biological functions. Poisonous in large quantities, nitric oxide nonetheless is produced in tiny amounts throughout the body.

In addition to relaxing blood vessels, scientists later found that the gas could help brain cells communicate with each other and immune cells kill disease-causing organisms, as well as assist the body's response to burns. From these basic discoveries, researchers are developing a number of treatments, including ones for high blood pressure, stroke, heart failure, complications of diabetes, and impotence.

The unique ability of nitric oxide to relax blood vessels, however, also intrigued researchers studying hypoxic respiratory failure. For reasons scientists do not fully understand, 2 out of every 1000 full-term infants suffer from this condition, which is actually a form of high blood pressure of the lungs. Blood vessels that would normally soak up oxygen-rich blood from the lungs remain tightly constricted so that too little oxygen is absorbed to keep the baby alive.

Most commonly, physicians first treat these infants by putting them on a ventilator to deliver 100 percent oxygen into the infants' lungs. If this is unsuccessful, physicians then turn to a procedure called extracorporeal membrane oxygenation (ECMO), which takes blood from a vein entering the heart and adds oxygen to it, before returning it to the body. ECMO, however, carries with it a 20 percent risk of permanent brain damage, and for every 1000 infants who receive ECMO therapy, only 82 percent survive.

Now, in the first conclusive trial of its kind, researchers from the NIH Neonatal Research Network have shown that using inhaled nitric oxide is an effective therapy for hypoxic respiratory failure in full-term infants who fail to respond to maximal conventional therapy, including100 percent oxygen. The study enrolled more than 200 infants suffering from hypoxic respiratory failure. Of those who received inhaled nitric oxide, about 46 percent needed ECMO therapy or died. In contrast, 64 percent of infants who only received the conventional therapy of 100 percent oxygen were referred for ECMO therapy or

had died. (The number of deaths among the two groups did not differ significantly, 14 percent for the nitric oxide group and 17 percent for the control group.)

When the infants were tested between 18 and 24 months of age, there was no increase in signs of damage to the brain or nervous system in the babies who received nitric oxide compared to those who received the standard treatment. Based on these studies, inhaled nitric oxide therapy for newborns suffering from hypoxic respiratory failure was approved by the Food and Drug Administration as an acceptable medical treatment. The NIH Neonatal Research Network is now conducting a trial to see if inhaled nitric oxide can also benefit premature infants with severe lung disease.

Solving the Puzzle of Inflammatory Bowel Disease

The inflammatory bowel diseases (IBD) known as Crohn's disease (CD) and ulcerative colitis (UC) affect nearly one million Americans. Typical symptoms of IBD include abdominal pain, fever, watery or bloody diarrhea, weight loss, and fatigue. Both forms of IBD are chronic illnesses that typically affect children and young adults and have major impact on their health and quality of life. Traditional therapy for IBD has consisted of immunosuppressive and anti-inflammatory drugs, antibiotics, and drugs to relieve the pain, fever, and other overt symptoms of the disease. Unfortunately, about one third of patients do not respond to medical treatment, and – in patients who do respond – remission is usually followed by relapse. Many patients ultimately require one or more surgeries to alleviate their symptoms.

Research is yielding new clues about the common final manifestation of IBD: chronic inflammation of the intestinal tract. The body's immune system is designed to identify and eliminate foreign invaders, generically termed "antigens." Inflammation is a complex response to an antigen, which includes increased blood flow to the affected region and an influx of cells to defend the body. This process is facilitated in part by the production of cytokines, proteins released by cells to alert the body to the presence of a threat and that may either promote or inhibit inflammation. Important pro-inflammatory cytokines include interferon-(IFN)-?, some members of the interleukin (IL) family, and tumor necrosis factor (TNF)-a. As the cells of the immune system destroy the antigen, the degree of the inflammatory response decreases and the injured area subsequently undergoes repair and recovery.

Under normal conditions, a balance exists between signals that promote inflammation and those that inhibit it. In patients who suffer from IBD, however, this balance is perturbed and pro-inflammatory signals predominate in the intestinal tract, leading to chronic inflammation and resultant tissue damage. While the trigger for this disturbance is unknown, it seems to arise from an abnormal reaction by the immune system to the bacteria normally present within the gut. A likely explanation for this aberrant immune response is that susceptible individuals inherit a genetic predisposition to IBD and possess an immune system unable to distinguish between benign and threatening stimuli. A major goal in the treatment of IBD is to induce and sustain remission over time, thereby limiting tissue damage and improving the quality of life for affected individuals. Drugs currently used to treat IBD fall into one of two general categories. One group acts quickly to relieve symptoms but is unsuitable for long-term use, owing to undesirable side effects. A second group of drugs is effective at maintaining remission over time, but is slow to act, thereby having limited usefulness in treating acute disease. Because of these limitations, researchers seek a fuller understanding of IBD at the molecular level, in the hope of identifying novel targets for new therapies.

Animal models of IBD have provided a wealth of new information about disease onset and progression. Historically, researchers induced IBD in animals by supplementing their diet with chemicals that irritated the lining of the bowel, producing symptoms reminiscent of human IBD. With the advent of molecular genetics, however, more sophisticated models of IBD have emerged. These models have revealed new insights into the origins of IBD and the roles played by pro- and anti-inflammatory cytokines. For example, mice engineered to overproduce TNF-a, a potent pro-inflammatory cytokine, exhibit severe

intestinal inflammation that closely resembles human Crohn's disease. Mice lacking IL-10, an anti-inflammatory cytokine, also develop widespread intestinal inflammation. Together, these findings indicate that disequilibrium in the balance between the levels of pro-and anti-inflammatory signals, resulting from either increased production of factors that promote inflammation or the absence of factors that inhibit it, can give rise to conditions that closely resemble IBD. Interestingly, in IL-10 deficient mice, the severity of IBD seems to be related to the presence of bacteria within the bowel, because mice housed in pathogen-free conditions or treated with anti-bacterial drugs develop more limited disease than do mice that are raised in a conventional environment. These studies support the hypothesis that IBD may arise from an inappropriate immune response, facilitated by a permissive genetic context, to otherwise benign environmental factors. The illness does not develop in genetically normal mice nor in mutant mice housed under special conditions.

These laboratory insights are currently being translated into novel therapies that target the molecular mediators of inflammation. Multiple clinical trials have examined the benefits of inhibiting pro-inflammatory stimuli or boosting the levels of anti-inflammatory signals in Crohn's disease. In these studies, the effectiveness of the experimental treatment is assessed using the Crohn's Disease Activity Index (CDAI), a numerical score that reflects multiple aspects of the disease. Scores of 200 to 400 indicate moderately active disease, while scores below 150 denote remission. "Clinical response" to treatment is usually defined as a decrease of 70 points or more in the index, which may not necessarily indicate remission. One strategy involves targeting TNF-a, perhaps the prototypic pro-inflammatory cytokine. In active Crohn's disease, a single injection of infliximab, an antibody that inactivates TNF-a, promotes a clinical response in two-thirds of patients and remission in approximately one-third. IL-10 has also been investigated as a potential therapy. In a small trial, disease activity scores were 50 points lower and remission rates were twice as high in Crohn's patients who received IL-10 for three weeks compared to those who did not.

As direct mediators of the immune response and inflammation, cytokines are obvious targets for novel IBD therapies. However, unexpected insights into the molecular causes of IBD have come from recent studies of the PPAR? gene, which is not a cytokine. It is a member of a family of proteins known as transcription factors that regulate which genes are turned on or off within a given cell. PPAR? was originally characterized as a protein that regulated metabolism and promoted the development of fat cells, and was investigated for its role in the development of diabetes. Surprisingly, cells of the large intestine also express PPAR?, where it can inhibit the production of pro-inflammatory cytokines. This discovery has led to the consideration of agents that activate PPAR?, and thereby reduce the levels of pro-inflammatory cytokines, as possible therapies for IBD. Several lines of experimental evidence support this reasoning. In a cell culture system, both naturally occurring and synthetic PPAR? activators inhibit the ability of intestinal cells to produce pro-inflammatory cytokines. Furthermore, in a mouse model of IBD, a significant decrease in severity of disease is noted when the mice are treated with synthetic PPAR? activators. These results suggest that therapies targeting PPAR? may be an effective component of an anti-IBD regimen. A pilot clinical trial, supported by NIH, is now under way to investigate this possibility.

All of these therapeutic strategies are designed to diminish the inflammatory response in order to relieve the symptoms of IBD. Future improvements in treatment of IBD, however, are likely to come from the identification of the genetic lesions that initially give rise to the disease. To identify genes that may be involved in a given disorder, scientists analyze DNA from genetically similar people, such as large families or members of relatively homogeneous ethnic groups, and look for a correlation between specific chromosomal segments and the occurrence of the disease. Using this approach, researchers have noted several genetic regions that seem to correlate with the development of IBD. The identification of multiple genetic loci on different chromosomes – some for Crohn's disease, some for ulcerative colitis, and others for both – suggests that there is unlikely to be a single underlying defect responsible for all forms of IBD. Although the identification of individual genes responsible for the development of IBD is years away, this information nevertheless represents an important first step in understanding the underlying genetic causes of IBD.

Ultimately, all these insights will be synthesized to provide a more complete base of knowledge about the causes of and potential treatments for IBD. While drugs focusing on cytokines such as TNF-a and IL-10 are effective in some patients, these novel therapies do not represent cures, because symptoms of the disease return after treatment is discontinued. To address this problem, researchers are currently conducting important work to investigate the possibility that combinations of agents – each targeting a different component of the immune response or a different facet of the disease – will prove more effective in the management of IBD than any single therapy. Critical insights into the origins of IBD will likely come from the identification of genes responsible for disease predisposition. In the future, these advances will lead not only to a clearer understanding of the disease, but also to new targets for drug development. Furthermore, genetic diagnosis should permit earlier detection of individuals at risk and should facilitate strategies to prevent the occurrence of IBD.

From Bench to Bone – Basic Research Yields Osteoporosis Treatments

A series of family photos documents the transformation of a beautiful young lady of 20 into a hunched and deformed woman of 60. The caption reads "My daily life has changed completely. I walk with two canes. I can't bend down and I am in constant pain. I cannot carry or pick things up and therefore I cannot do my own shopping. All my life I have been active, now I can't even do routine activities."

The human costs of osteoporosis are enormous. This disease is characterized by low bone mass and bone deterioration. A comprehensive treatment program includes a focus on proper nutrition, exercise and safety issues to prevent falls that may result in fractures. In addition, a medication may be prescribed that will slow or stop bone loss and increase bone density. If left untreated, the bones of a person with osteoporosis become weak and fragile, leading to an increased risk of fractures. All bones may become brittle, but fractures to the hip, spine and wrist occur most often and can lead to prolonged or permanent disability, even death. According to the National Osteoporosis Foundation (NOF), approximately 10 million people in the U.S. have osteoporosis and about 18 million more have low bone density, placing them at increased risk for developing the disease. Osteoporosis has been reported in people of all ethnic backgrounds and the chances of developing osteoporosis are four times greater in women. The NIH Cost of Illness Report estimates national direct and indirect expenditures for osteoporosis and related fractures at $14 billion each year, and the cost is rising.

Although there is no cure for osteoporosis, in the past thirty years, major strides have been made in its treatment. Several new classes of agents have been approved by the Food and Drug Administration that help to stop bone loss and prevent further fractures. Bone cells have been shown to respond to the female hormone estrogen, which acts to slow down the removal of calcium from bone. Unfortunately, estrogen, when taken alone, has been shown in some studies to slightly increase a women's risk of developing breast and endometrial cancer. SERMs, or selective estrogen receptor modulators, were developed to maximize the beneficial effects of estrogen on bone while minimizing the adverse effects on other organs and tissues. Bisphosphonates are another class of agents that specifically target bone and act to reduce bone loss by mimicking the action of estrogen. Research on the regulation of bone development and remodeling has played a pivotal role in fueling the major scientific advances that are expanding understanding of the development and progression of osteoporosis and that are leading to newer and more effective strategies for its treatment and prevention. Scientific advances open exciting and promising new avenues for future research exploration and provide more options for treatment.

Bone is a living, growing tissue. It is made mostly of collagen, a protein that provides an elastic framework, and calcium phosphate, a mineral that adds strength and serves to harden the framework. Hormones play a major role in the regulation of bone formation and bone loss. The amount of bone mineral present in bone at any given time reflects the net difference between these two processes. One key hormone responsible for regulating the cells involved in bone formation – the osteoblasts – is parathyroid hormone (PTH). Paradoxically, PTH exhibits both anabolic and catabolic effects – that is, it has been shown to control the transfer of calcium both into and out of bone. A pioneering researcher isolated and purified PTH. This accomplishment led to the collaborative development of a novel

radioimmunoassay to measure PTH levels in the blood. Using the radioimmunoassay, researchers could then study the factors governing PTH secretion. Resulting data clearly demonstrated that changes in serum calcium, in turn, controlled PTH secretion. The isolation and purification of PTH paved the way for further studies on the synthesis of recombinant PTH and on the mechanisms of action of this hormone.

A few years later, researchers announced the successful identification, cloning and sequencing of the cellular receptor for PTH in several species, including rat, mouse and humans. Although the receptor's overall structure greatly resembled that of other trans-membrane peptide hormone receptors, it differed sufficiently so as to constitute a major new receptor subfamily. Once the structure was deduced, it was then possible to analyze its molecular mechanism of action. Such knowledge is crucial to understanding the processes that lead to weakened bones and to the development of hormone-based therapies.

It had long been known that the amount of circulating calcium played a role in regulating the amount of PTH secreted. Yet, until recently, the exact mechanisms that permitted the cells of the parathyroid gland to secrete hormone in response to calcium levels remained unknown. Impressive studies led to the identification and cloning of a calcium-sensing receptor, defining an important step in the regulatory pathway of calcium-responsive PTH secretion. This receptor acts to "sense" extracellular levels of calcium and "signals" the parathyroid gland to regulate levels of PTH secretion. Different parts of the calcium sensing receptor structure were shown to mediate different parathyroid gland signaling pathways, explaining how secreted PTH can at times signal the osteoblast to increase bone mineral, and at other times, signal the osteoclast – a cell involved in the breakdown of bone – to reduce bone mineral. Thus, this research helped to explain the mystery of the anabolic and catabolic effects of PTH. The long-term implications of this work suggested the possibility of not only designing therapeutic agents that would suppress the signaling events leading to bone loss, but of agents that would stimulate the formation of bone, such as synthetic PTH.

An understanding of the anabolic actions of hormones such as PTH led to the design of two small-scale clinical trials testing the efficacy of synthetic PTH as a treatment for osteoporosis. Results demonstrated a beneficial effect on increasing spinal bone mineral density and preventing bone loss from the hip and total body in young women. Synthetic PTH is also proving beneficial in treating a severe form of osteoporosis caused by glucocorticoid hormones, such as prednisone, which are used to treat inflammation. An ongoing clinical study is evaluating the effect of daily administration of synthetic PTH on the risk of fractures in postmenopausal women who have glucocorticoid-induced osteoporosis and are currently receiving estrogen replacement therapy. Preliminary results indicate a greatly reduced risk of spine fractures and non-traumatic, non-spine fractures within one to two years of beginning therapy. Other recent research has shed light on how estrogen works to maintain bone mass by shortening the lifespan of the cells that are responsible for resorption of bone mineral. It does so indirectly, by stimulating the production and release of a potent growth factor in bone. In the absence of estrogen, this control is lost. The osteoclasts are no longer properly regulated, resulting in excessive bone loss.

Though no significant side effects have been attributed to PTH therapy, current treatment requires painful injections, which the patients must perform daily. Thus, the development of an oral agent that exhibits few side effects would provide a valuable treatment alternative. A number of studies are under way on novel oral agents that enhance PTH secretion in animal models. These agents act through the calcium-sensing receptor, allowing PTH secretion to be regulated independently of calcium levels in the blood. Preliminary results from a few studies have demonstrated a dramatic decrease in the kind of bone turnover that results in excessive loss of bone mineral, thus suggesting additional potential therapeutic agents for the future treatment and prevention of osteoporosis and related bone disorders.

The long-term investment in studies of the underlying mechanisms of bone loss and bone remodeling, as well as the development of cellular and animal models to study these processes, has enabled the development of new drugs to treat osteoporosis. These approaches have laid the foundation for understanding the mechanisms of action of these agents and for the design of safe and more effective therapies. These discoveries open exciting and promising new avenues for future exploration and provide more options for the treatment of this disease. A research imperative is to capitalize on these discoveries to develop newer, safer and more effective compounds to prevent and treat osteoporosis. With translation of these novel treatments to medical practice, it will become possible to prevent bone loss, reduce the incidence of fractures, and improve the overall quality of life for patients at risk for osteoporosis.

Interleukin-2 Receptor Targeting as Therapy for Sight-threatening Uveitic Disease

Intraocular inflammatory disease, or uveitis, is a commonly seen ocular disorder that mainly affects children and young adults. It has been estimated to cause about 10% of the severe visual handicap in the United States and if untreated can rapidly lead to blindness. Some inflammations may be due to an infectious agent. These diseases include toxoplasmosis and cytomegalovirus retinitis (a complication of AIDS). However, a large number of intraocular inflammatory conditions appear not to be caused by an infectious agent but rather by an altered immune response of the body to itself, known as autoimmunity. Animal models for these diseases, which are termed endogenous or autoimmune uveitis, have helped enormously in dissecting this abnormal immune response we see in patients and to evaluate candidate therapies.

The present therapy for these non-infectious uveitic disorders are drugs that suppress the immune response (immunosuppressive). While they can be very useful in suppressing the immune response, there are significant side effects in terms of toxicity as well as suppression of beneficial immune responses to microorganisms. The goal of vision scientists has been to better understand the underlying mechanisms that lead to autoimmunity and ocular inflammation, so as to more specifically turn off only the harmful response, and try to do so with minimal or no side effects. We have seen that one central mechanism in uveitis appears to involve a particular population of white blood cells or lymphocytes known as T cells. These cells carry the "immune memory" and are able to "orchestrate" inflammatory responses in the body. One of the major ways they orchestrate these inflammatory responses is to produce mediators of inflammation called interleukins and growth factors, of which a number have been identified, with more to be discovered. One of the most important is interleukin-l (IL-l), which plays a central role in recruiting and perpetuating the immune response and serves as a growth factor to the T cells themselves. On the surface of activated T cells are molecules or receptors. These receptors interact with IL-1 and are crucial for cell survival. This makes them a good target for therapy. NIH intramural scientists decided, therefore, to target the IL-l receptor, as a means of eliminating the harmful T cells that are involved in disease.

The approach of therapeutically targeting the IL-l receptor of activated T-cells was initially evaluated in 1989 in a rodent model of endogenous uveitis, which has many characteristics of the disease seen in humans. This was done using a genetically engineered toxin fused to IL-l, and was found effective. However, due to its toxicity it was not considered a safe therapy for humans. The next step was to prepare a monoclonal mouse antibody to the human IL-l receptor. A "humanized" version of this antibody dubbed HAT (for Humanized Anti-T-activated) and given the trade name Zenopax, was initially evaluated in a non-human primate model of uveitis that shares even more similarity with the human disease than do rodent models. The therapy was very effective in both preventing the development of the experimental uveitis and in treating the disorder once it appeared. Zenopax was then used in an open clinical trial to treat ten patients with severe sight threatening uveitis who had to be treated with potent immunosuppressive medications to keep their disease in check. Nine of the ten patients were able to be gradually taken off their immunosuppressive medication(s) and continued to be treated with Zenopax alone. The disease appears to remain under control with infusions of Zenopax

given once a month. No other group of patients has received this therapy for so long a period, and to date, we have not identified any side effects that can be attributed to the experimental medication. It has been the impression of the clinicians and the perception of the patients that the quality of their lives has improved with this new approach to treating their disease.

This innovative therapy is an example of the ability of the NIH's intramural program to bring ideas from the laboratory bench to the bedside, and the synergy that can be achieved by close collaboration between different Institutes. The success of this therapy in treating uveitic disease has demonstrated the potential for this therapy in the field of autoimmune diseases in general, and its use is now being actively pursued by many groups in various areas of medicine, e.g., neurology and dermatology. The first 10 uveitis patients in the original study continue to be followed to further the understanding of long term effects of this therapeutic approach. A parallel study has begun to evaluate this therapy in the treatment of Behçet's disease, a rapidly progressing, blinding disease that often requires aggressive immunotherapy to stem the ocular complications. Additionally, preparations are now underway to apply this approach to children that need systemic immunotherapy for their uveitis. Planning has also begun for a multicenter study to define better the potential indications for this new approach to treat sight-threatening diseases.

Alzheimer's Disease Amyloid: Discovery of Molecular Processes Leads to New Therapeutic Approaches

Alois Alzheimer first described the plaques and tangles found in the brains of dementia patients in 1906. His patient died at age 55 and for the next 60 years scientists and clinicians thought that this form of early-onset dementia was very different from the dementia of old age. Indeed, dementia in older people was considered an almost inevitable part of aging. Then, in the 1970s, scientists discovered that older persons with dementia often had exactly the same plaques and tangles in their brains as those described so clearly by Alzheimer.

Late-onset Alzheimer's disease (AD) is, in fact, the major form of this disease, responsible for up to 4 million cases of dementia in the U.S. alone. As our population ages, there could be up to 14 million cases by the year 2050. Clearly, unless a treatment is found, AD will impose a tremendous and increasing burden on both our health care system, and the many family members who care for AD patients over years of cognitive and physical decline.

Two abnormal structures in the brain are hallmarks of AD: amyloid plaques and neurofibrillary tangles. Plaques are dense deposits of protein and cellular material outside and around the brain's nerve cells. Tangles are twisted fibers that build up inside the nerve cells. Though scientists have known about plaques and tangles for many years, more recent research has revealed much about their composition, how they form, and their possible roles in the development of AD.

The plaque amyloid is considered to be a critical factor because its deposition is thought by many to trigger the cascade of events leading to the pathology found in the brains of AD patients. Amyloid may therefore turn out to be a critical target for eventual treatment. Building on years of research funded by the NIH, private foundations and industry, researchers have discovered that injecting amyloid into mice or administering it nasally actually causes an immune reaction that stops plaques from developing in brain. If this "immunization" strategy works in humans, it could provide the first effective treatment for AD.

How did this potential breakthrough come about? In the 1980s, amyloid from the brain tissue of an AD patient was found to consist of a short protein fragment (a peptide). The likely DNA sequence coding for this amyloid peptide was predicted from its amino acid sequence and was then used as a molecular probe to search the 100,000 or so genes present in human DNA for the specific gene that produces amyloid. Several research groups found the gene, naming it the amyloid precursor protein (APP) gene.

Three mutated genes causing major forms of inherited early-onset AD were identified by the mid-1990s: the APP gene itself as well as genes named presenilin 1 and presenilin 2. These discoveries have initiated the modern era of AD research, for study of the proteins made by these genes and their metabolic pathways has provided major biological clues to the sequence of events in the development of AD pathology. Understanding these pathways and gene products is allowing for the first time the design of treatments that are targeted to the basic and early processes that underlie the pathology of AD.

These interventions have the potential to arrest disease before it affects brain function and causes symptoms.

A major advance was developing the first animal models of AD by inserting mutated human APP genes into mouse embryos and observing formation of amyloid plaques and other AD-like pathologies in the brains of these "transgenic" mice as they age. Since then, numerous transgenic models have been developed, allowing scientists to understand better how a complex array of intercellular pathways can interact to affect the production of AD plaques. Importantly, these transgenic animal models also provide a means of testing the efficacy of different treatments on reducing build up of plaques and on cognitive function.

Much research has focused on amyloid as accumulating evidence suggests that it may be possible to prevent AD from developing by interfering with amyloid production or its aggregation into plaques. Using both transgenic animal models and experiments in tissue culture, scientists have succeeded in understanding much about how amyloid is snipped out of APP, how amyloid aggregates into plaques, and how plaques might lead to the brain destruction of later stages of AD. Importantly, these discoveries are also facilitating discovery of ways in which plaque production may be slowed. Many leads are being pursued, including development of compounds to halt amyloid deposition at various steps along the pathway, or to prevent downstream harmful effects.

During the past year, scientists provided evidence that one of the proteins (enzymes) that snip amyloid out of APP may actually be identical to one of the genes, presenilin 1, whose mutations can cause inherited early onset AD. Now, more effective drugs to inhibit production of amyloid by this enzyme can be developed. Prototypes are being developed and at least one is already being tested for safety in industry-sponsored trials.

The recent papers describing a potential vaccination treatment for preventing plaque formation excited AD researchers. An industry scientist attempted this unconventional approach and surprisingly it worked in mice. Transgenic mice that make human amyloid in brain tissue were used in this study. An amyloid solution was injected into the mice. They readily developed an immune reaction to the injected amyloid; and amazingly, if mice were immunized repeatedly for many months, plaque development was all but halted. This breakthrough has been successfully replicated in different strains of transgenic mice by a number of NIH-funded laboratories. Importantly, one recent report shows that nasal inhalation of the amyloid can also retard plaque production. This route of delivery may be better tolerated than repetitive injections.

The results of these experiments were startling. Perhaps vaccination could work similarly in humans, halting development of AD long before clinical deterioration sets in? Both technical and theoretical hurdles need to be overcome. On a practical level, the human immune system is rather different, so it is not certain that injection of amyloid will produce the same immune response, attacking amyloid and arresting plaque formation. Would there be harmful side effects? Preliminary industry-sponsored human safety trials have shown no harmful effects of one vaccine injection and trials looking at effects of

multiple injections are ongoing. Will an intervention that prevents plaque formation indeed have an effect on the neuronal death and symptoms of AD? A report at the recent Alzheimer's disease World Congress showed encouraging data that vaccination prevented cognitive decline associated with amyloid production in a transgenic mouse model. While such findings are very promising, only clinical trials in humans will determine whether the vaccine approach can safely prevent the clinical signs of AD or stop its progression.